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## TRANSLATING SCIENCE INTO SURVIVAL

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# *Abstracts* BOOK

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# POSTER SESSION A

## Combination therapies with immune checkpoint blockers

### A001 / Clinical activity of BEMPEG plus NIVO observed in metastatic TNBC: preliminary results from the TNBC cohort of the Ph1/2 PIVOT-02 study

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Background: Recent advances in the treatment of metastatic triple-negative breast cancer (mTNBC) include the use of checkpoint inhibitor (CPI) therapy. CPIs, in combination with chemotherapy, has been shown to prolong survival in mTNBC pts with PD-L1 positive baseline status. However, CPI monotherapy is less active, with objective response rates (ORR) ranging from 5% (Adams, Dirix) to 10% (Emens). ORR with CPI monotherapy also appears to be lower in PD-L1 negative baseline pts, ranging from 0% (Emens) to 5% (Adams), as well as in ≥2L pts, with reported ORR of 6% (Emens). Another reported negative predictive factor for CPI benefit in these pts includes prior taxane (Schmid). Thus, more effective CPI treatment combinations are needed, particularly in pts with PD-L1 negative tumors. The safety and clinical activity of bempegaldesleukin (BEMPEG or NKTR-214), a CD-122 preferential IL-2 pathway agonist, plus the anti-PD1 CPI, nivolumab (NIVO) was evaluated in PIVOT-02, a multicenter Phase 1/2 study in multiple solid tumor settings and includes a cohort of pts with mTNBC. PIVOT-02 recently reported promising preliminary clinical activity and safety data for pts with metastatic melanoma and urothelial cancers. This is the first report of BEMPEG plus NIVO in mTNBC.

Methods: 43 pts with mTNBC received BEMPEG (0.006 mg/kg) + NIVO (360 mg) q3w. Pts were categorized by line of therapy (1L, 1L but early relapse [defined as metastatic relapse within 12 months of last chemotherapy in EBC setting], 2L, ≥3L). In addition to PD-L1 status, other predictive or prognostic clinical factors were assessed, including the following five: age, disease-free interval, LDH, number and type of metastatic sites, and prior taxane. Response was assessed every 3 cycles by RECIST v1.1. Per protocol, ORR was evaluated in the 38 pt efficacy-evaluable population, defined as pts with at least 1 post-baseline tumor assessment (5 were not evaluable: 3 clinical progressions, 2 deaths due to progressive disease after 1 cycle). Baseline immunohistochemistry (IHC) analysis for PD-L1 was performed using 28-8 pharmDx (Dako) and defined as PD-L1 negative (<1% tumor cell expression) and PD-L1 positive (≥1% tumor cell expression). Safety and tolerability were assessed by adverse event (AE) analysis reported by CTCAE v4.03.

Results: 42 of the 43 patients reported at least 1 of the 5 poor prognostic or negative predictive clinical factors for CPI benefit, with a median of 2. Baseline factors that indicated a poor prognosis population include: young age (21% ≤ 40 years old), 51% PD-L1-negative, 65% who had received 2 lines or more treatment in the metastatic setting, and 40% had elevated LDH. Lung and liver were the most common sites of metastasis occurring in 46.5% and 23.3% respectively, and 44% of all pts had ≥3 metastatic organ sites. ORR in the 38 efficacy-evaluable pts was 13% (5/38), regardless of PD-L1 status, with a disease control rate (defined as complete response or partial response or stable disease for at least 8 weeks) of 45% (17/38). ORR was 11% (2/19) in PD-L1 negative and 17% (2/12) in PD-L1 positive pts. Pts with 2 lines or more of prior therapy had an ORR of 20% (5/25), regardless of PD-L1 status. Treatment-related Grade 3/4 AEs occurred in 23% of pts, with dehydration, hypotension, and myalgia as the most frequently reported (4.7% each), consistent with previous reports.

Conclusion: Clinical activity was observed in mTNBC pts treated with BEMPEG plus NIVO, notably in pts with poor prognostic features or negative predictive clinical factors for CPI benefit, including negative PD-L1 status. Additional efficacy analyses, including ORR, duration of response, and biomarker analyses, will be presented. These data support future development of this doublet in mTNBC pts who are PD-L1 negative at baseline, in combination with chemotherapy. This study (NCT02983045) and registrational studies in other solid tumor settings are ongoing.

Keywords: BEMPEG, NKTR-214, Triple-negative breast cancer, Nivolumab.

## Immunotherapies, cell based

### A002 / Skewed T cell distribution in pancreatic cancer patients

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To understand potential immune system alterations in newly diagnosed, untreated, pancreatic cancer patients and provide a foundation for immunotherapy, we profiled PBMC from pancreatic ductal adenocarcinoma (PDA) patients and age matched healthy controls using high dimensional CyTOF analysis. We developed two immune profiling panels : a broad profiling panel that includes 45 phenotypic markers that together permit the identification and enumeration of the main innate and adaptive immune cell subsets in humans, and a deep profiling panel that includes 45 features focusing on T cell phenotype and biology. We report a 2-fold increase in monocytes, and more plasmocytes in circulation in pancreatic cancer patients compared to age-matched controls. Using high dimensional approaches, we observe skewed T cell differentiation in pancreatic cancer patients, with CD8 T cells biased towards more CD45RA-positive CD27-positive CCR7-positive CD95-positive CD49d-positive stem cell memory cells (P=3x10-4), more CD45RA-negative CD27-positive CCR7-negative effector memory cells (P=0.002) and less CD45RA-positive CD27-negative CCR7-negative late effector memory cells (P=0.01) than age-matched controls. We further examined alterations of T cell differentiation in CD8 T cell compartment in human spleens, and report increased proportions of late effector memory T cells in pancreatic cancer patients as compared to age-matched controls. Comparable observations of increased numbers of intra-splenic effector T cells were made in genetically engineered (KPC mice : LSL-KrasG12D/+; LSL-Trp53R172H/+; Pdx-1-Cre), as well as orthotopic mouse models of pancreatic cancer. In the former, accumulation of effector T cells into the spleen was also observed for adoptively transferred listeria-experienced T cells. Together, these results demonstrate a trafficking defect of effector T cells in the context of pancreatic cancer at baseline. We are now investigating the mechanisms underlying these observations, as well as their impact on T cell immunity of the patients. Our goal is to understand the nature of the skewing and how any changes in baseline immune health of the T cell compartment related to disease progression and/or response to therapy. These studies should provide a foundation for improving therapy in pancreatic cancer patients. Additionally, they should provide new insights into the heterogeneity of T cell differentiation.

Keywords : Pancreatic cancer, T cell differentiation, Baseline immunity, Trafficking.

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Morrison, Trends in Cancer 2018

## A003 / Engineering therapeutic T cell circuits that harness combinatorial antigen recognition to overcome tumor heterogeneity in glioblastoma

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Treatment of solid cancers with chimeric antigen receptor (CAR) T cell is challenging because of a lack of target antigens that are both tumor-specific and homogeneously expressed in the cancer cells. In glioblastoma (GBM), the epidermal growth factor receptor variant III (EGFRvIII) antigen is highly tumor-specific but is a non-ideal target because it is heterogeneously expressed. In contrast, several more homogenously expressed antigens, while associated with GBM, are non-ideal targets because they are also expressed in other normal organs. We show that “prime-and-kill” dual antigen recognition circuits can overcome this challenge in vitro and in vivo. Where a synthetic Notch (synNotch) receptor that recognizes a specific but heterogeneous antigen (EGFRvIII) is engineered to locally induce expression of a CAR that kills neighboring cells expressing tumor-associated antigens. This type of circuit spatially integrates recognition of multiple antigen targets across the tumor, yielding T cells with improved effectiveness in treating heterogeneous GBM.

Keywords : Tcell, Glioblastoma, Heterogeneity, Chimeric Antigen Receptor.

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## A004 / Development and implementation of the first-in-human clinical trial of CAR-NKT Cells

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Introduction : Due to their natural anti-tumor properties and ability to localize to the neuroblastoma (NB) tumor site, Vα24-invariant natural killer T cells (iNKTs) are promising candidate immune effectors for chimeric antigen receptor (CAR)-based immunotherapies targeting NB and other solid tumors. In pre-clinical studies iNKTs expressing a CAR construct containing an optimized GD2-specific single-chain variable fragment with the CD28 costimulatory endodomain and IL-15 (CAR.GD2.IL-15) demonstrated superior in vivo persistence and anti-tumor activity in a xenogenic NB model. These results provided rational for clinical testing of iNKTs expressing CAR.GD2.IL-15 in NB patients.

Experimental procedures : Following validation of current good manufacturing practice (cGMP) protocols for the generation and clinical scale expansion of CAR-iNKTs, we initiated a phase 1 clinical trial to assess safety, persistence and efficacy of autologous CAR-iNKTs in relapsed/refractory NB (NCT03294954) using 3+3 dose-escalation design. To date, four cell therapy products have been manufactured and two patients received the infusion of 3x106 CAR+iNKTs/m2 following lymphodepletion. One of these patients received a second infusion at the same dose level.

Summary of the data : Patient derived iNKTs were successfully expanded to produce ≥109 CAR-iNKTs within 9-17 days, resulting in 95.43% iNKT purity (median, range : 81.2 – 97.17%) and 44.52% CAR expression (median, range 20.18-81.2%). Lymphodepletion-induced hematologic toxicities were detected but patients

did not experience CAR-iNKT-related toxicity. Both patients treated showed evidence of CAR-iNKT expansion in perpherial blood peaking at week 3 post-infusion. Flow cytometry analysis of cells from a tumor biopsy showed evidence of CAR-iNKT trafficking to a metastatic lesion and to bone marrow. One patient had stable disease four weeks post-injection. Another patient with two established metastatic bone lesions prior to therapy experienced complete regression of a right femur lesion and a nearly complete regression of a large sternum lesion as determined by MIBG-SPECT imaging at 4 and 8 weeks post-infusion.

Conclusion: Initial clinical results indicate that adoptively transferred autologous CAR.GD2.IL-15 iNKTs are safe, can expand in vivo, localize to the tumor site and induce regression of metastatic lesions in heavily pretreated, relapsed/refractory neuroblastoma patients.

Keywords: Natural Killer T cells, Neuroblastoma, CAR, IL-15.

## A005 / Engineering T cell circuits that can sense antigen density with ultrasensitive threshold

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T cells can be engineered to kill tumor cells via synthetic T cell receptors known as chimeric antigen receptors (CARs), this approach is becoming a highly promising therapeutic strategy for cancer treatment. However, current CAR T cells fail to discriminate between cells expressing high and low levels of common antigens which limits its use against solid tumors. Potential target antigens, such as Her2, are over expressed in tumors but often also found on normal cells at lower expression levels, and CAR T cells get activated even by low amounts of target antigen resulting in potentially deadly toxic effects. We are approaching this problem of antigen density discrimination by engineering T cell circuits that can sense antigen density with ultrasensitive threshold.

Standard CAR T cells are highly sensitive to even low densities of the target antigen. Current strategies for generating CAR T cells consist of selecting antibodies with high affinity since previous studies have shown that the CAR T cell activity is inversely correlated with the antibody's affinity. Recent studies suggest that lowering the receptor affinity on a CAR T cell could increase its selectivity against target cells with different antigen densities. We have explored the effects of CAR expression and antibody affinity for antigen density discrimination. Overall, changing CAR affinity or expression leads to linear changes in antigen density response curves. For ultransensitive response, we have designed a two-step recognition-activation circuit that involves two receptors. In this circuit an initial recognition event alters the potency of a subsequent response. We combined a weak affinity receptor that turns on the expression of a high affinity receptor that fully activates the cell. For these circuits we have made used of a new class of modular receptors called synthetic notch receptors (SynNotch). SynNotch receptors use antibody-based domains to recognize a target antigen, but when activated, they control transcription via a cleaved transcriptional domain. We have designed and expressed several synNotch/CAR circuits and have tested their ability to achieve antigen density sensing. These circuits use a synNotch receptor to control the expression of a CAR — all recognizing the same Her2 ligand. The constructs are introduced into T cells via standard lentiviral infection, and sorted for receptor expression using flow cytometry. We took the best circuit candidate and performed studies in mouse models. We tested either conventional CAR T cells or our new engineered T cells in bilateral tumors into NSG immunocompromised mice, in which one side contains a low density Her2 K562 tumor, and the other side contains a higher density Her2 K562 tumor. The engineered T cells are injected into the tail vein, and allowed to traffic freely to either tumor. We monitored tumor size by caliper measurements over the course of 4 weeks, and track the differential clearance of the high and low density tumors. We have confirmed that when using a fixed affinity standard CAR, then T cells kill both high and low expression level Her2 tumors with equal efficiency. This is in contrast with T cells expressing our recogniton-activation circuit that show sharp discrimination between target cells with normal and disease levels of Her2, both in vitro and in vivo. This work illustrates the power of engineering response circuits over engineering only receptors for cell therapeutics.

Keywords: T cell engineering, Synthetic biology.

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## A006 / Improving chimeric antigen receptor T-Cell potency by repurposing endogenous immune pathways

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CAR T-cell therapies hold great promise for treating a range of liquid malignancies but are however challenged to access and eradicate solid tumors. To overcome this hurdle CAR T-cell were engineered to secrete different cytokines known to improve T-cell antitumor activity, prevent T-cell anergy and reduce activation induced cell death. While cytokine-expressing CAR T-cell were shown to be be highly active against solid tumor in in vivo models, they have also led to toxicity associated with the systemic release of cytokine. Therefore, new engineering strategies enabling the fine tuning of cytokine secretion by CAR T-cell are warranted.

We sought to explore one these engineering strategies by integrating an IL-12 chimeric heterodimer expression cassette under the control of the endogenous promoters regulating PD1 or CD25. Because both genes are known to be activated upon tumor engagement by CAR T-cells, they could be repurposed to secrete cytokine only in the vicinity of a given tumor. This approach would reduce the potential side effects induced by their systemic secretion while maintaining their capacity to improve antitumor activity.

By combining TALEN technology with AAV6 repair vectors delivering the CAR to the TRAC locus and the IL-12 to the CD25 or PD1 loci, we have engineered CAR and IL-12 expressions under the respective control of TCR and CD25 or PD1 regulatory elements. This double targeted insertion led to the disruption of PD1 and TRAC genes, to the expression of an anti-CD22 CAR and to the conditional secretion of IL-12 in the media. Such secretion was found to be transient, dependent on tumor engagement and to follow the regulation patterns of CD25 or PD1 genes, commonly observed upon T-cell activation.

Activation-dependent secretion of IL-12 enhanced proliferative capacities of CAR T-cells as well as their antitumor activity, both in in vitro models and in vivo xenografts. The complexity of such gene editing approach did not impair the genomic integrity of the cells.

This proof of concept paves the way for seamless multi-repurposing of immune pathways to generate smarter CAR T-cells able to sense and react to their environment in a highly regulated and specific manner.

Keywords: IL-12, CAR, TALEN, Synthetic biology.

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## A007 / CAR-T cells with neutrophil-activating protein induce bystander immunity and eradicate solid tumors with heterogenous expression of the CAR target antigen

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Chimeric antigen receptor (CAR) T cell therapy has been remarkably successful for B-cell malignancies, while many challenges still exist for efficient CAR-T cell therapy of solid tumors. They include (i) endothelial and stromal barriers, which block CAR-T cell infiltration, (ii) local immune suppression within solid tumors, which dampens the activity of CAR-T cells, and (iii) intra- and intertumoral heterogeneity in antigen expression leading to clonal escape upon CAR-T cell treatment. Herein, we harness an efficient immune modulator from the pathogenic world, the neutrophil-activating protein (NAP) from Helicobacter Pylori, to arm CAR-T cells and thereby increase their potency. In vitro, we show that NAP secreted from CAR(NAP)-T cells upon tumor cell recognition recruits innate immune cells such as neutrophils and monocytes and promotes dendritic cell (DC) maturation and polarization towards a T helper type-1 immune response. The induced DCs in return potentiate CAR(NAP)-T cell activity, leading to enhanced cytotoxicity capacity. In vivo, we show that CAR(NAP)-T cells regress solid tumors and prolong survival of mice significantly more efficient than conventional CAR-T cells. Importantly, when solid tumors where 50% of the tumor cells expressed the CAR target antigen were treated by CAR(NAP)-T cells, the bystander immune activation induced led to tumor growth arrest and eradication of "mixed tumors" in the majority of treated mice. Conventional CAR T-cells did not result in "mixed tumor" eradication in any mouse treated. CAR(NAP)-T cell-treated tumors had increased infiltration of DCs, neutrophils, inflammatory monocytes and CD107a+ cytotoxic T cells. Endogenous anti-tumor T cell activity, induced by the CAR(NAP)-T cell treatment, was evaluated by using tumors expressing both the CAR target antigen and ovalbumin as a model tumor antigen. We observed epitope spreading and increased infiltration of both effector and central memory-like cytolytic ovalbumin-specific T cells when CAR(NAP)-T cells were used. Taken together, our data show that NAP secreted from CAR(NAP)-T cells creates a proinflammatory tumor microenvironment, induces DCs to support CAR(NAP)-T cell killing, and recruits endogenous CD8+ T cells and innate immune cells with the ability to kill tumor cells, including tumor cells not expressing the antigen targeted by the CAP(NAP)-T cells. The results indicate that NAP-secretion from CAR-T cells can improve treatment efficacy for solid tumors and warrant this to be evaluated in a clinical study.

Keywords: CAR T cell, bystander immunity, antigen heterogeneity, neutrophil-activating protein.

## A008 / CAR T cell therapy of solid tumors – the characteristics of the scFv matters

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In recent years, CAR T cell therapy has been successfully implemented against B cell malignancies. Despite encouraging results against hematological malignancies, CAR T cell therapy against solid tumors has been less stunning. This is in part due to the complexity of the solid tumors with antigen-heterogeneity and an immunosuppressive microenvironment but can also be due to an imperfect CAR design. It is evident that an optimal CAR design must be developed to generate a robust anti-tumor immune response. The CAR construct consists of an extracellular antigen-recognizing domain, usually a single chain variable fragment (scFv) from an antibody, a hinge region and a transmembrane domain that link the extracellular part to the intracellular activation domains. Small changes in any of these elements can have great impact on therapeutic efficacy and so far, no universal implications have been found.

Glioblastoma is a malignant brain tumor with a median survival of only 15 months despite harsh treatment regimen. New therapeutic strategies are needed to improve outcome for patients and CAR T cell therapy has shown encouraging results. We aim to develop novel scFv constructs targeting interleukin 13 receptor alpha 2 (IL13Rα2), which is over-expressed by large part of glioblastomas, for CAR T cell therapy. Five scFvs were selected and used to create CAR T cells based on their affinity to IL13Rα2, both as a pure protein and expressed on target cells. All five CAR T cell constructs displayed significant proliferation upon co-culture with target expressing glioblastoma cells compared to control CD19 CAR T cells. In addition, all candidates specifically killed target cells in a dose dependent manner in vitro. However, there were extensive variations in both cytotoxic capacity and proliferation rate between the constructs upon target cell encounter, that appeared not to be directly linked to affinity.

During ex vivo expansion using, CD3, CD28 and CD2 antibodies, the percent of CAR+ T cells in the culture decreased significantly within 7 days for 2 out of 5 CAR T cell constructs. Interestingly, the ex vivo expansion pattern could reflect proliferative and killing capacity of CAR T cells upon target cell encounter. It has been previously established that scFvs can induce clustering of CAR molecules on the T cell surface and thus give rise to antigen-independent tonic signaling. We hypothesize that our scFvs induced tonic signaling to various extent and that reflect cytotoxic capacity and viability of CAR T cells.

In conclusion, we highlight the importance of evaluating several scFvs in a CAR T cell setting to identify the best candidate for treatment of solid tumors. We have found three candidates against glioblastoma that are persistent during expansion and demonstrate efficient cytotoxic capacity in vitro. These constructs will be further evaluated to identify the top candidate.

Keywords: CAR T cells, single chain variable fragment (scFv), glioblastoma, solid tumors.

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## A009 / Intra-cavitary infusions of autologous dendritic cell/cytokine-induced killer cells in the treatment of refractory malignant pleural effusions and ascites: A Chinese population-based cohort study

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*Purpose:* To explore the safety and efficacy of intracavitary infusion of autologous dendritic cell (DC)-cytokine-induced killer (CIK) cells in advanced cancer patients with malignant pleural effusions or ascites.

Methods: DC-CIKs (mean yield of 1.36×109 (range, 0.74~4.98×109 cells) were expanded ex vivo from peripheral blood mononuclear cells obtained by apheresis and were infused three times into the pleural or peritoneal cavity. The volume of malignant effusions was assessed radiologically. Peripheral blood subsets were enumerated by flow cytometry. EORTC QLQ-30 was assessed with cancer related instrument during the DC-CIK treatment.

Results: Thirty-seven patients with breast, lung and other malignancies were enrolled. The intracavitary DC-CIK infusions (16 intrapleural and 21 intraperitoneal) were well tolerated with no grade 3/4 adverse events. There was one complete effusion response (3%), 13 partial responses (35%), 12 stable diseases (32%) and 11 progressive diseases (PD) (30%) resulting in a clinical effusion control rate (CCR) of 70% (26/37). The infused number of total CIKs and the CD3+/CD8+ and CD8+/CD28+ T cell subsets were associated with effusion control (p=0.013). Moreover, increased peripheral blood CD3+/CD8+ (P=0.035) and decreased CD4+/CD25+ T cells (P=0.041) following the CIK infusions were associated with malignant effusion and ascites control.

Conclusion: Intracavitary autologous cellular immunotherapy could control malignant pleural effusions and ascites and was associated with peripheral blood immunological T cell recovery.

Keywords: autologous T cell immunotherapy;, Malignant pleural effusion;.

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## A010 / Development of human-derived synthetic Notch receptor augmented CAR T cells against solid tumors

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The chimeric antigen receptor (CAR) T-cell immunotherapy platform can achieve incredible success when treating certain leukemias. However, its effective adaptation to other cancers, especially solid tumor cancers, remains a frontier in translational research. The paucity of ligands unique to solid tumors and the heterogeneous ligand expression on solid tumors pose key challenges for CAR T-cell immunotherapy. Furthermore, while desirable ligand targets may be overexpressed on solid tumors, these ligands are often expressed on healthy tissue and their recognition by CAR T cells can result in potential adverse reactions that manifest as on-target/off-tumor toxicities. We seek to expand the clinical applicability of CAR T-cell immunotherapy for treating solid tumor cancers by creating "smart" CAR T cells that are armed only in the solid tumor microenvironment to address these functional challenges. This combinatorial activation of CAR T cells is enabled by the recently developed synthetic Notch (synNotch) receptor that can be modularly designed to recognize a desired ligand and consequently trigger the transcription of the CAR transgene. Specifically, we are evaluating a humanized synNotch receptor as a clinically-relevant tool to regulate CAR-mediated function against EGFR-expressing solid tumors. Although the EGFR ligand is broadly overexpressed on many solid tumors and is a desirable target as a key driver of the cancer phenotype, it is also expressed on healthy tissue. Using solid tumor model systems to demonstrate this combinatorial approach, we aim to restrict anti-EGFR CAR expression to the solid tumor environment with a humanized synNotch receptor that recognizes a solid tumor target. Model Jurkat and primary T cells are genetically modified by Piggybac-mediated transposition to ectopically express the humanized synNotch receptor and its regulated cargo (reporter protein or CAR). These modified T cells are co-cultured in vitro with target expressing tumor cells to confirm the functionality of our humanized synNotch receptors (quantified by flow cytometry and cytokine production assays). The functional synNotch-reporter/CAR combinations are then tested in vivo with NSG immunocompromised mice implanted with solid tumors. To date, we have verified the in vitro functionality of each component that comprise a humanized synNotch receptor recognizing a solid tumor target and its regulated anti-EGFR CAR. We have finalized a genetic design with these components that facilitates our production of engineered CAR T cells containing this humanized synNotch receptor and its responsive payload. While still in progress, this work ultimately broadens the future designs of clinical CAR T-cell immunotherapies directed against solid tumor cancers.

Keywords: CAR T cell, synNotch.

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## A011 / Cord-blood stem cell-derived dendritic cells specifically originate from CD115-expressing precursors

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Dendritic cells (DCs) are professional antigen presenting cells, which instruct both the innate and the adaptive immune system. Once mature, they provide all necessary signals to activate and prime naïve T-cells. These characteristics makes them excellent candidates for vaccination. Intervention strategies aiming at manipulation of DCs require in-depth insights in their development and functional properties. DCs can be generated from CD34+-cells. CB is a good source to provide CD34+-stem cells for the generation of Cord blood (CB)-derived DC. We demonstrate that CD34+ CB -derived stem cells develop in several types of myeloid progenitor cells. Recent advances in state-of-the-art techniques led to significant insights into DC ontogeny. Isolation of the DC progenitor cells by flow cytometry, enables precise characterization, using RNA sequencing, to clear which progenitor drives the development of DCs. Here we show that CD115+-progenitor cells are responsible for the generation of the CB-DCs. Functional assays demonstrate that CD115-derived DCs are highly mature upon stimulation, migrate efficiently and possess a high capacity to stimulate tumor-antigen-specific T-cells. The discovery of a committed DC precursor in the CB-derived stem cell culture further enables optimized utilization of DC-based vaccine to provide powerful anti-tumor activity and long-term memory-immunity, e.g. in combination with hematopoietic cell transplantation (HCT)

from the same CB graft to overcome relapse in refractory cancer patients.

Keywords: Cord Blood, Dendritic cells, Vaccine, Stem cell transplantation.

## A012 / Optimization of a GMP-grade large-scale expansion protocol for Cytokine-Induced Killer cells using gas-permeable static culture flasks

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Cytokine-Induced Killer (CIK) cells are ex vivo expanded T cells with NK cell phenotype. They express both CD3 and CD56 antigens, and exert a potent antitumor activity against a variety of tumors. Several clinical trials demonstrated the safety and the feasibility of CIK cell therapy, with very low side effects and minimal graft-versus-host toxicity. In this study, we developed a GMP-compliant protocol for robust large-scale expansion of CIK cells using G-Rex® gas-permeable static culture flasks. CIK cells were obtained by stimulating healthy donor PBMCs with GMP-grade IFN-γ, IL-2 and CD3 mAbs, and were cultured in G-Rex6® or G-Rex®6M well plates. CIK cells in G-Rex6® were split only once at day 7 to reduce cell density, whereas the number of CIK cells culterd in G-Rex®6M was not adjusted. In both culture conditions, fresh IL-2 was provided every 3-4 days. We compared these two culture protocols with the culture in standard flasks. Phenotype was analyzed by flow cytometry and cytotoxicity was assessed against several tumor cell lines by calcein-release assay. CIK cells cultured in G-Rex6® well plates showed an outstanding cell expansion compared to G-Rex®6M well plates or standard culture flasks, with a 400-fold expansion and a mean of 109 total cells obtained per single well in 14 days, starting from just 2.5x10^6 cells per well. Moreover, the cultures in G-Rex6® were characterized by an higher percentage of CD3+CD56+ cells, as compared to G-Rex®6M or standard culture flasks (38.6314.9%, 35.0315.5%, 24.0317.8%, respectively). Cells cultured in all devices had a comparable expression of NKG2D, NKp30, NKp44, 2B4 receptors. Importantly, CIK cells expanded in G-Rex®6 were as cytotoxic as cells expanded in standard culture flasks. Conversely, CIK cells cultured in G-Rex®6M showed a remarkable reduction of cytotoxicity against tumor cell targets, thus suggesting that cell density during expansion could affect CIK cell activity. We propose a GMP-compliant protocol for robust large-scale production of CIK cells. G-Rex® system allows to obtain large amounts of CIK cells highly enriched in the CD3+CD56+ subset and endowed with high cytotoxic activity; this can be accomplished with just a single cell culture split at day 7, which dramatically reduces the culture manipulation as compared to the standard culture flasks. Notably, this strategy can be further and easily scalable to produce CIK cells for clinical immunotherapy applications.

Keywords: Adoptive cell therapy, Cytokine-Induced Killer cells, GMP-grade culture, large-scale expansion.

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## A013 / Adoptive cell therapy of hematological malignancies using Cytokine-Induced Killer cells retargeted with monoclonal antibodies

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Cytokine-Induced Killer (CIK) cells are a population of effector cells that represent a promising tool for adoptive cell therapy. From a technical and methodological point of view, they are easily expandable ex-vivo and safe, and demonstrated efficacy against hematological malignancies. CIK cells exert cytotoxicity against a broad range of tumor histotypes but not against normal tissues and hematopoietic precursors. We recently reported that they have a relevant expression of FcγRIIIa (CD16a), which can be exploited in combination with clinical-grade monoclonal antibodies (mAbs) to redirect their cytotoxicity in an antigen-specific manner, to improve their antitumor activity. Indeed, the engagement of CD16a on CIK cells leads to a potent antibody-dependent cell-mediated cytotoxicity (ADCC) against ovarian cancer both in vitro and in vivo. Based on this observation, we investigated whether CIK cells could be specifically retargeted against B-cell malignancies by combination with anti-CD20 mAbs, namely Rituximab® and Obinutuzumab®. CIK cells were obtained from peripheral blood mononucleated cells of healthy donors, and stimulated in vitro with IFN-γ, CD3 mAb (OKT3) and IL-2 for 14 days; fresh IL-2 was provided every 3-4 days. CIK cell phenotype was analyzed by multicolor flow cytometry; cytotoxic activity was assessed by calcein AM-release assay against EHEB (CLL), Raji (Burkitt lymphoma), RCK-8, TMB-8, Karpas 422 (DLBCL) tumor cell lines, and primary samples obtained from CLL and DLBCL patients after written informed consent. The combination with either the mAbs significantly increased CIK cell cytotoxicity not only against lymphoma cell lines, but also against the primary tumor samples. Depletion of NK cells from CIK cell bulk cultures did not affect target cell lysis, thus confirming that the antibody-mediated increase of cytotoxicity was mainly accountable to the CIK cell fraction. Taken together, these data indicate that the combination treatment with CIK cells and anti-CD20 mAbs could represent a novel approach for the treatment of hematological malignancies, alternative to the use of chimeric antigen receptor (CAR)-T cells.

Keywords: Adoptive cell therapy, Cytokine-Induced Killer cells, B-cell malignancies, Monoclonal antibodies.

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## A014 / Oncolytic virus derived type I interferon restricts CAR T cell therapy

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While chimeric antigen receptor (CAR) modified T cells have been remarkably successful in hematologic malignancies, the comparatively modest results in solid tumors derive from multiple immune suppressive mechanisms in the microenvironment that limit CAR T infiltration, persistence and function. Oncolytic viruses (OVs) replicate preferentially in cancer cells compared to normal healthy cells. OVs initiate targeted tumor infection while simultaneously expressing therapeutic transgenes (such as cytokines, or tumor antigens) to augment anti-tumor immunity. Infection initiates a pro-inflammatory cascade that stimulates the production of chemokines and cytokines that alter the recruitment of inhibitory and activating immune cells. Our preclinical testing of VSV encoding IFNb has led to the clinical evaluation of the platform in diverse indications, both as a monotherapy and with checkpoint inhibitors. Based on our studies, the highly inflammatory nature of oncolytic viruses and their ability to remodel the tumor microenvironment suggested to us that they would provide a complementary mechanism of action to both recruit and potentiate the functionality of CAR T cells. Despite inducing a robust pro-inflammatory shift in the cytokine and chemokine profile of the tumor, VSVIFNb infection was associated with attrition of murine third generation EGFRvIII CAR T cells in the B16EGFRvIII model. We demonstrate both a T cell intrinsic, as well as CAR T cell specific, effect of oncolytic virus derived type I interferon which promoted apoptosis, activation, and inhibitory receptor expression. Interferon receptor knockout CAR T cells were largely refractory to VSV- associated attrition and provided combinatorial therapy with VSVmIFNb. Our study highlights the need for evaluation of multiplexed CAR strategies in systems which are fully immunocompetent, and provides rationale for the modification of CAR T cells to insulate them against oncolytic virus-derived "heat."

Keywords: CAR T cell, Oncolytic virus.

## A015 / Novel epitope derived from PBK (PDZ-binding kinase), a cancer testis antigen, allows CD8+ T cell recognition of pediatric brain tumors

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Currently, treatment options for pediatric brain cancers are limited to surgery, radiation, and chemotherapy, often with limited results. With the development and early success of T cell-based immunotherapy for adult patients with various cancers, our goal is to develop immunotherapy for pediatric brain tumor patients. However, the mutational burden of pediatric cancers is low, limiting the scope of immunotherapy targeting neoantigens. We hypothesized that targeting the aberrant upregulation of cancer testis antigens (CTAs) would allow for T cell recognition of tumors with low mutational burden. Our biobank, published by the Olson group in Nature Medicine in 2018, contains paired genomic and whole exome sequencing, RNA expression data, and methylation profiling for a variety of primary pediatric brain tumor samples, as well as samples xenografted into mice or adapted to grow in tissue culture. We performed an in silico analysis of CTA expression in these pediatric brain tumor samples to determine CTAs with increased expression that might be suitable targets for T cell-based immunotherapy. We found that PBK (PDZ binding kinase), a CTA, is highly upregulated in pediatric brain tumor samples compared to normal brain tissue. Using HLA binding prediction algorithms and a reverse immunology screening approach, we found that rare CD8+ T cells from the repertoire of normal healthy donors could lyse autologous target cells pulsed with a PBK-derived nonamer peptide predicted to bind HLA-A*02:01, a prevalent HLA allele. We isolated PBK epitope-specific CD8+ T cell clones from multiple donors (3 out of 4 tested to date) indicating potent immunogenicity of the neoepitope, and found the clones used various nonpublic T cell receptor sequences. Importantly, CD8+ T cells specific for this novel epitope could recognize and kill pediatric brain tumor cell lines in vitro, indicating endogenous processing and presentation of the PBK-derived peptide. Specifically, 4 of 4 HLA-A*02:01+ brain tumor samples were killed in vitro, including 2 high grade glioma samples and 2 atypical teratoid rhabdoid tumor (ATRT) samples. Using a panel of lymphoblastoid cell lines, we tested the HLA-restriction of our PBK-derived nonamer and confirmed that clones recognized PBK peptide presented by HLA-A*02:01. Studies on the efficacy of A*02:01-restricted, PBK-specific CD8+ T cells against brain tumor burden in vivo are planned, using pediatric patient-derived tumor xenografted into mice in flank or cerebellum. Using the same screening approach, we have also recently isolated CD8+ T cells that recognize two additional novel PBK-derived epitopes predicted to bind HLA-B*07:02, and further characterization of these T cell lines is ongoing. As PBK is expressed across a wide variety of pediatric brain tumor samples, including those with particularly poor prognoses like high grade gliomas, these initial results offer a promising first step towards the development of immunotherapy options for many pediatric brain tumor patients.

Keywords: Immunotherapy, Pediatric brain cancer, Cancer testis antigen.

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Brabetz et al. Nature Medicine Volume 24, pages 1752–1761 (2018).

## A016 / Isolation of neoantigen-specific T cell receptors from different human and murine repertoires

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Mutation-specific T cell receptor (TCR)-based adoptive T cell therapy presents a promising immunotherapeutic strategy for

solid tumors with the potential for long-term recognition and eradication of malignant cells. Specificity of a mutation-specific TCR can be transferred to autologous T cells and thus engineered tumor-reactive T cells can be generated in sufficient numbers for therapeutic applications. The isolation of mutation-specific TCRs, however, is time consuming and laborious. Here, we exploited different TCR repertoires to optimize isolation strategies to generate high-avidity TCRs for potential clinical applications. Within the frame of the Berlin Institute of Health (BIH)-CRG study "Moving mutation-specific T cells into the clinic", non-synonymous somatic mutations of cancer patients were identified. Resulting tumor-specific altered peptide sequences were ranked according to their predicted binding score to the patient's HLA-molecules. These selected neoepitope candidates were used to stimulate peripheral blood lymphocytes (PBL) of either the patient, or HLA-matched healthy donors in an in vitro culture system using peptide loaded autologous antigen-presenting cells (APC). Simultaneously, for HLA-A*02:01 positive patients, HLA-transgenic mice containing the human TCR repertoire were immunized with the same peptide to generate high affinity TCRs. TCR sequences were identified from both, human and murine peptide-reactive CD8+ cells. TCR gene cassettes were constructed for TCR gene transfer into primary human lymphocytes. Neoantigen recognition was assessed in co-culture assays of transduced lymphocytes and APC by measuring T cell activation, degranulation and cytokine secretion. Four patients were selected for in vitro stimulation cultures and neoepitope-reactive T cells could be isolated for two patients. For one colon carcinoma patient, stimulation cultures of patient derived CD8+ T cells did not generate neoepitope-reactive T cells. However, reactive T cells could be isolated from the murine repertoire and one HLA-matched healthy donor with reactivity against three out of 16 neoepitope candidates and functional TCR gene rearrangements were identified. Interestingly, TCRs from immunized HLA-transgenic mice were specific for two different neoepitopes as the TCR originating from the healthy donor repertoire. Additionally, neoepitope candidates used to generate specific TCRs from one healthy donor repertoire did not result in the generation of neoepitope-reactive T cells in other donor repertoires. Whether this is primarily a phenomenon of immunodominance, related to inherent characteristics of the epitope, the MHC class haplotypes of the donor or an inherent poor capacity of a given T cell repertoire to respond to a particular peptide-MHC complex needs to be further investigated. Additionally, as a limiting experimental factor, low precursor frequencies could also result in a low detection rate of neoepitope-specific T cells in different healthy donor repertoires.

Keywords: adoptive T cell therapy, mutation-specific TCR, neoepitope, in vitro stimulation.

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## A017 / T cells secreting nanobody-based immune checkpoint inhibitors

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Cancer therapy is experiencing a paradigm shift due to the clinical success of immune checkpoint inhibitors (ICI), yet efficacy remains limited to a few tumor types and a minority of patients. One of the main determinants of clinical response to ICI is the tumor infiltration by anti-tumoral lymphocytes. The main side effects are autoimmune toxicities related to self-directed CD8 T cell activation upon ICI treatment. These side effects become almost prohibitive when using ICI combination. Our project aims at bypassing the limitations of ICI systemic treatment by performing adoptive cell transfer (ACT) of tumor-targeting lymphocytes transduced for the secretion of anti-PD-L1 nanobodies. Nanobodies offer the advantage of a good stability and penetration ability at the tumor site while displaying a very short half-life in the blood stream due to renal filtration. Altogether, our approach could improve tumor infiltration by lymphocytes thanks to the adoptive transfer, increase the tumoral exposure to anti-PD-L1 treatment, while minimizing the side effects related to systemic exposure. We constructed viral vectors containing either the sequence of an anti-human or an anti-mouse PD-L1 nanobody. We obtained production and secretion of human and mouse nanobodies by human and mouse lymphocytes respectively. We confirmed the binding capacity of the nanobodies on PD-L1 positive cells and their blocking capacity on the PD-1/PD-L1 interaction. We established an in vivo mouse model of ACT in Balb/c mice. We successfully extracted tumor infiltrating lymphocytes (TILs) from CT26 tumor-bearing mice and expanded them in vitro. We proved the killing activity of these TILs against CT26 tumor cells in vitro. We also plan to evaluate our approach in the B16-Ova model as well as a human xenograft model in NSG mice.

In conclusion we are now ready for the generation and evaluation of anti-PD-L1 nanobody-secreting CD8 T cells. We are going to evaluate in vitro the T cell proliferation, killing activity and cytokines secretion ability. In vivo we will perform adoptive transfer of nanobody-secreting T cells and will evaluate both their therapeutic efficacy as well as their secretion ability at the tumor site. In perspective, this approach can be further developed to generate T cells secreting multiple therapeutic molecules, including a combination of different nanobody-based ICI or a combination of an ICI with a cytokine or chemokine. This local delivery approach, by avoiding systemic toxicity, broadens the possibilities of immunotherapeutic combinations compared to the classical systemic administration.

Keywords: Nanobody, PD-L1, Adoptive cell transfer, Toxicity.

## A018 / Incessant ER stress responses promote dendritic cell dysfunction in ovarian cancer

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Harnessing the intrinsic ability of our immune system to recognize and eliminate malignant cells represents the most promising anti-cancer therapy since the development of chemotherapy. However, hostile tumor microenvironmental conditions inhibit the optimal activity of protective immune cells. Targeting immunosuppression and re-programming immune cell function in the tumor microenvironment are fundamental requirements for developing successful cancer immunotherapies. The central hypothesis of this study is that ovarian tumors trigger endoplasmic reticulum (ER) stress and aberrant activation of the IRE1α-XBP1 pathway in infiltrating DCs to cripple key immuno-metabolic processes that are necessary for the development of protective T cell responses. To determine molecular mechanism how IRE1α-XBP1 overactivation defines regulatory DC phenotypes in the ovarian cancer microenvironment, we generated bone marrow-derived DCs (BMDC) from conditional KO (IRE1α, PERK and ATF6) mice selectively and independently deleting each ER stress sensor in immune cells. Interestingly, this extensive genetic analysis revealed that IRE1α-XBP1 is the dominant ER stress sensor to provoke overexpression of immunosuppressive and tumorigenic factors such as Arginase1, IL-6, Cox-2 and Vegf-A in DCs undergoing ER stress. We also identified that tumor-associated DCs lacking IRE1/XBP1 exhibit defective production of the immunosuppressive bioactive lipid PGE2, which correlated with decreased proportions of Foxp3+ regulatory T cells (Tregs) at tumor locations in mice bearing ovarian cancer. These data revealed that transferred DCs lacking ER stress sensor IRE1/XBP1 not only demonstrate enhanced capacity to elicit anti-tumor T cells in situ, but also reduced PGE2-driven tolerogenic capacity that prevents Treg accumulation at tumor sites. Since therapeutic DC-based vaccines have shown limited effects in ovarian cancer patients, we tested the novel translational hypothesis that XBP1-deficient BMDCs would be better equipped to endure and function in the tumor microenvironment when used as therapeutic vaccines. To test this hypothesis, we implanted mice with ID8 ovarian carcinoma cells and tested the effects of BMDC-based therapeutic vaccination in the presence or absence of treatment with cisplatin. At advanced stage of disease progression, and in the absence of prior cisplatin, only therapeutic vacci-

nation with XBP1-deficient BMDCs increased host survival, while vaccination with their WT counterparts did not induce anti-tumor effects. Our results provide a unique mechanistic rationale for targeting the IRE1-XBP1 arm of the ER stress response to reprogram and enhance anti-tumor immune cell function in cancer. These findings could pave the way for devising a new generation of cancer immunotherapies that may improve the dismal survival of >21, 000 American women affected by ovarian cancer each year.

Keywords: Endoplasmic Reticulum (ER) stress, Ovarian cancer, Dendritic cell.

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## A019 / γ-secretase inhibition increases efficacy of BCMA-specific chimeric antigen receptor T cells in multiple myeloma

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B cell maturation antigen (BCMA) is a validated target for chimeric antigen receptor (CAR) T cell therapy in multiple myeloma (MM). Despite promising objective response rates, the majority of patients relapse and BCMA down-modulation has been suggested as a probable escape mechanism. A potential obstacle to BCMA-directed CAR T cell efficacy is cleavage of BCMA from the tumor cell surface by the ubiquitous multisubunit γ-secretase complex. BCMA cleavage reduces ligand density for CAR T cell recognition and releases a soluble BCMA (sBCMA) fragment. Sufficient sBCMA can accumulate in the bone marrow of MM patients to inhibit CAR T cell recognition of tumor cells, and potentially limit efficacy of BCMA-directed adoptive T cell therapy. The aim of this project is to investigate whether blocking BCMA cleavage by small molecule γ-secretase inhibitors (GSIs), originally developed for the treatment of Alzheimer's, could augment BCMA-targeted CAR T cell therapy. We found that exposure of myeloma cell lines and patient samples to GSIs markedly increased surface BCMA levels in a rapid dose-dependent fashion, concurrently decreased sBCMA concentrations, and improved tumor recognition by CAR T cells in vitro. GSI treatment of MM tumor bearing NOD/SCID/γc-/- mice increased BCMA expression on tumor cells, decreased sBCMA in peripheral blood, and improved antitumor efficacy of BCMA-targeted CAR T cell therapy. Importantly, we also demonstrate that short term GSI administration to MM patients markedly increased the percentage of BCMA+ tumor cells, and upregulated BCMA surface expression in vivo. Based on these data, an FDA-approved clinical trial (NCT03502577) has been initiated, combining GSI with concurrent BCMA CAR T cell therapy.

Keywords: CAR T cell therapy, BCMA, Multiple myeloma, gamma secretase inhibitor.

## A020 / The role of Fc-receptors in NK-mediated immunity against cancer and virus infection

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The innate branch of the immune system plays a pivotal role in initiating an appropriate immune response to early disease. Natural killer (NK) cells are amongst the first line of defense against pathogens and cancer. Recent studies have also demonstrated that NK cells can possess immunological memory. Fcgr3, more commonly known as the CD16 receptor binds to antibodies on tumor or pathogen-infected cells resulting in potent activation of NK cells and the release of cytotoxic granules that kill pathogenic cells, a process known as antibody-dependent cellular cytotoxicity (ADCC). In humans, memory NK cells induced by cytomegalovirus infection display augmented CD16-mediated ADCC function against antibody-coated tumors. To address this in an in vivo model, we are using various transgenic mouse lines to determine the importance of Fc-gamma receptors in the control of pathogenesis by NK cells. We are investigating how CD16-mediated responses influence the control of mouse cytomegalovirus (MCMV) and if they plays a role in generating anti-CMV NK memory. In addition, we are investigating the importance of CD16 responses on NK in a cancer immunotherapy model using anti-CD20 antibody treatments to B cell lymphoma. The findings from these studies will expand our knowledge of NK cell biology and will give insights on how to more effectively engage NK cells in cancer treatment.

Keywords: Natural killer cells, Lymphoma, Fc receptors, immunotherapy.

## A021 / Genome-Wide CRISPR screens reveal SOCS-1 as a dominant intrinsic checkpoint of CD4 T cells quorum sensing

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What determines the intensity and length of the CD4 T cell response is incompletely known. As MHC-II peptide complexes persist several weeks in vivo, disappearance of antigen (Ag) does not explain the prototypic contraction phase observed at day 6-8 of any primary responses. We previously showed that direct Ag-specific T-T interactions rapidly exclude Ag-experienced CD4 T from an ongoing immune response in a mechanism reminiscent of a quorum-sensing system (1). The molecular mechanisms had not been deciphered. Using an in vivo genome-wide KO screen, we could restore the proliferation of Cas9-expressing Ag-specific memory CD4 T cells and identified SOCS-1 (suppressor of cytokine) as the major non-redundant molecule involved in this cell-intrinsic dominant inhibition. We are now characterizing the probably redundant messengers (cytokines), sensors and transducers (receptors) governing Ag-specific CD4 T cell collective behavior. These findings have important consequences for vaccinations strategies and adoptive cancer immunotherapies.

Keywords: Genome-wide engineering, primary T cells, intrinsic checkpoint.

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## A022 / Protransduzin™ - A novel transduction enhancer to accelerate clinical scale CAR-T Production

Michael Drosch (JPT Peptide).

The advent of cellular immunotherapies using genetically reprogrammed immune cells such as T-lymphocytes with chimeric antigen receptor (CAR-Ts) or engineered T-cell receptors (TCR-Ts) has led to promising new treatments for cancers. However, due to the complex multi-step manufacturing process, these therapies are still very costly and currently approved therapies exceed 400k US$ per patient. In order to make CAR-T immunotherapy more affordable, a reduction of the production-related cost of goods and process optimization is highly desirable. A crucial manufacturing step is the actual gene transfer of CARs and TCRs which to date mostly relies on lenti- or retroviral vectors and often results in low transduction rates. In the past years a range of genetic transfer enhancers is being used to improve transduction with variable success. These include amphiphilic macromolecules, cationic polymers, and adhesion molecules. Until now the coating of cultureware with recombinant fibronectin (or a fragment thereof) has been shown to provide fair enhancement and

is widely accepted. However, coating procedure is cumbersome with time consuming liquid handling steps in a cleanroom environment. Here we describe Protransduzin™, a novel peptide that can enhance transduction of CARs in human leukapheresis-derived CD3 lymphocytes by up to 45% over control dependent on culture system and multiplicities of infection. A side-by-side comParison of Protransduzin™ with recombinant fibronectin and an additional, commercially available histidine-rich amphipathic peptide showed that Protransduzin™ is at least as potent as the adhesion molecule but more potent than the other peptide. Since Protransduzin™ can be added directly with the viral particles it can be used to streamline CAR-T production. Especially in connection with mini bio-reactors it is possible to do an en gros production by allowing transduction and expansion in one vessel. Thanks to our optimized protocol shown here hands-on time can be reduced along with the contamination risk arising from repeated liquid handling steps.

In conclusion, using Protransduzin™ reduces CAR-T cell production costs and is a significant step towards making cellular therapies more affordable in future.

Keywords: Transduction, CAR-T, Retrovirus, Cellular Therapies.

## A023 / Effects of hypoxia on human T cell phenotype and function

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The tumor microenvironment (TME) is frequently characterised as immuno-suppressive, attenuating the anti-cancer immune response. Hypoxia is characteristic of many solid tumors and can influence both the origination and outcome of an anti-tumor immune response via multiple mechanisms, many of which involve expression of the hypoxia-induced transcription factor, HIF-1α on both tumor and immune cell compartments. Primary in vitro assays are frequently used to profile the activity of novel targeted agents pre-clinically. However, these assays are predominantly performed under normoxic conditions which may not represent those conditions found in the TME. As such we have investigated how hypoxic conditions impacted the activity of primary human T cells stimulated with Staphylococcal Enterotoxin B. T cell function in normoxic conditions (21% oxygen) and hypoxic conditions (1% oxygen) were compared including how activation under normoxic conditions (simulating lymph node activation) followed by culture in hypoxic conditions (simulating migration to the tumor) would impact T cell activity. Observations included differences in cytokine production from T cells cultured in hypoxia compared to normoxia. These results indicated a significant upregulation of both IL-2 (p=0.0015) and IL-12p70 (p=0.0275) in hypoxia, while IFNɣ (p=0.0005), IL-13 (p=0.0412), IL-4 (p=0.0030), IL-5 (p=0.0049), GM-CSF (p=0.0005), IL-18 (p=0.0001), IL-21 (p=0.0059), IL-22 (p=0.0075) and IL-23 (p=0.0007) were significantly downregulated. Cytokine production from T cells stimulated under normoxia and then moved to hypoxia more closely resembled the profile observed from T cells stimulated under hypoxia. We observed a significant decrease in the proliferation of CD4+ (p=0.0001) and CD8+ T cells (p=0.0002) activated in hypoxia. Furthermore, hypoxia resulted in the downregulation of activation markers including CTLA-4 (p=0.0005 & p=0.0006) and PD-1 (p=0.0002 & p=0.0070) on CD4+ and CD8+ T cells respectively. Despite this downregulation, our data demonstrate that the activity of a combination of anti-CTLA-4 and anti-PD-1 monoclonal antibodies was maintained in hypoxic conditions. However, the magnitude of the IFNɣ response following PD-1/CTLA-4 blockade is attenuated under hypoxic versus normoxic conditions. Taken together, these data show clear differences in T cells stimulated in hypoxia compared to normoxia. Furthermore, a combination of PD-1 and CTLA-4 targeted antibodies are active under hypoxic conditions. However, pre-clinical studies should empirically determine the activity of novel targeted agents under hypoxic conditions in order to provide a more physiologically and translationally relevant indicator of activity in the clinical setting.

Keywords: Hypoxia, T cell, PD-1, CTLA-4.

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## A024 / Suppression of GvHD and augmentation of GvL by allogeneic human double negative T cells and underlying mechanisms

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a potentially curative treatment for hematological malignancy patients. However, disease relapse and graft-versus-host disease (GvHD) remain as unresolved issues. We recently demonstrated that ex vivo expanded human CD3+CD4-CD8- double-negative T cells (DNTs) induce anti-leukemic activity without attacking normal cells and fulfill the requirements of “off-the-shelf” therapy in pre-clinical models. However, whether ex vivo expanded human DNTs can suppress GvHD without hampering graft-versus-leukemia (GvL) response has not been studied. In this study, the potential of ex vivo expanded human allogeneic DNTs as an “off-the-shelf” adoptive cell therapy to suppress GVHD while enhancing GvL effect and their underlying mechanisms of action have been investigated. To elucidate the immunosuppressive activity of ex vivo expanded DNTs conventional T (Tconv) cells were stimulated polyclonally or using allogeneic antigens in the presence or absence of DNTs. To determine whether DNTs can suppress GvHD, xenogeneic GvHD models were used, where NSG mice were infused with human Tconv cells with or without DNTs, and the severity of GvHD was assessed through monitoring mice and histological analysis of GvHD-prone tissues. To investigate the mode of action, potential molecules involved in DNT-mediated immunoregulatory activities were identified through high-throughput screenings of DNT surface molecules, and functional involvements of the expressed molecules were determined via blocking assays. To investigate whether DNTs simultaneously suppress GvHD while inducing GVL, leukemia-bearing mice were treated with Tconv cells with or without DNTs, and the leukemia engraftment level and normal tissue damage were assessed. Tconv cells proliferated significantly less when stimulated in the presence of DNTs, and DNTs inhibited priming of Tconv cell to allo-antigens, developing resistance to host-versus-graft rejection. In GvHD-xenograft models, mice co-infused with DNTs showed enhanced survival with less severe tissue damage. Further, Tconv cells obtained from DNT-co-treated mice were less activated than those infused with Tconv cells alone. High throughput screening of expressed surface molecules identified an array of molecules expressed on DNTs, and involvement of several molecules in DNT-mediated Tconv cell suppression was functionally confirmed by blocking studies. Importantly, DNT infused with Tconv cells into leukemia-bearing mice simultaneously targeted leukemia and suppressed GvHD without compromising the GvL activity of Tconv cells, resulting in a higher leukemia clearance rate and less severe tissue damage than infusion of Tconv cells alone.

In conclusion, this study demonstrates the potential of “off-the-shelf” DNTs as an adjuvant therapy to allo-HSCT to prevent GvHD while inducing GvL in patients and identified the underlying mechanism of DNT-mediated GVHD-suppression.

Keywords: adoptive T cell therapy, allogeneic hematopoetic stem cell transplantation, leukemia, graft versus host disease.

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## A025 / Preclinical development of CD37CAR T-cell therapy for treatment of B-cell lymphoma

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T cells modified to express chimeric antigen receptor (CAR) targeting CD19 (CD19CAR) have produced remarkable clinical responses in patients with relapsed/refractory B-cell acute lymphoblastic leukemia. CD19CAR T-cell therapy has also demonstrated notable efficacy in B-cell non-Hodgkin lymphoma (B-NHL) patients. However, a subset of patients who relapse after CD19CAR T-cell therapy have outgrowth of CD19– tumor cells. Hence, development of alternative CARs targeting other B-cell markers represents an unmet medical need for B-cell acute lymphoblastic leukemia and B-NHL. Here, we confirmed previous data by showing that B-NHL has high expression of CD37. A second-generation CD37CAR was designed, and its efficacy in T cells was compared with that of CD19CAR. In vitro assessment of cytotoxicity and T-cell function upon co-culture of the CAR T cells with different B-cell lymphoma target cell lines demonstrated comparable efficacy between the two CARs. In an aggressive B-cell lymphoma xenograft model, CD37CAR T cells were as potent as CD19CAR T cells in controlling tumor growth. In a second xenograft model, using U2932 lymphoma cells containing a CD19– subpopulation, CD37CAR T cells efficiently controlled tumor growth and prolonged survival, whereas CD19CAR T cells had limited effect. We further showed that, unlike CD19CAR, CD37CAR was not sensitive to antigen masking. Finally, CD37CAR reactivity was restricted to B-lineage cells. We are also working on several design modifications, mainly focused on the hinge size in order to study the impact on the overall CAR functionality. Collectively, our results demonstrated that CD37CAR T cells can effectively eradicate B-cell lymphoma tumors when CD19 antigen expression is lost and support clinical testing in patients with relapsed/refractory B-NHL.

Keywords: CAR-based therapy, Immunotherapy.

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## A026 / The investigation of personalized immunotherapy targeting neoantigen for liver, pancreas, and biliary tract cancer

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In recent, the clinical benefits of immune checkpoint inhibitor and adoptive immunotherapy with T cells suggested that neoantigens derived from passenger gene mutation might cause anti cancer immune response in vivo. Unfortunately, the effect of immune checkpoint inhibitors is less than 20% in most solid cancers (1-5). Moreover, the cancer types in which adoptive immunotherapy with T cells is effective are also limited. In particular, the effect on gastrointestinal cancer has been still unclear, while various passenger gene mutations have been reported (6-7). Since passenger mutations are usually individual in each patient, personalized immunotherapy should be required to achieve the maximum response by cancer immunotherapy (8-9).Here, to explore the possibility of personalized immunotherapy against hepatocellular carcinoma (HCC), pancreas adenocarcinoma (PDAC), biliary tract cancer (BD), and liver metastasis (Meta), we try to identify tumor-reactive CTLs from tumor infiltrating lymphocytes (TILs), and to determine the neoantigens recognized by tumor-reactive CTLs.

Methods: Total 105 patients who were received radical resection in our hospital (National Cancer Center East, Japan) until Dec. 2016 to Jan. 2018 were enrolled to this study. DNA and RNA were harvested from both normal and tumor tissues to analyze the mutation burden by WES. Refer to the results of omics analysis, the prediction of neoantigens was performed by netMHCpan Ver.4. In 59 patients of these (HCC=22, PC=13, and Meta=24), the reactivity of TILs against autologous tumor were successfully assessed by in vitro degranulation assay (CD107a assay). Using Chromium (10x Genomics), TCR repertoire and transcriptome of primary T cells were analyzed by single cell levels.

Results: When PD-1+CD8+ T cells were co-cultivated with autologous tumor, we detected the CD107a+ fraction in half of patients, however, the % of CD107a were always less than 1 %. Similar results were obtained by the analysis of TCR repertoire of primary PD-1+CD8+ T cells in tumor tissue. Comparing the % of CD107a+ in each patient, we found that the % of CD107a+ CD8+ TILs tended to higher in HCC patients than that in others (median =0.084% in HCC, =0.027% in Meta, =0.019% in PC). The multi-fluorescence immunohistochemical analysis of HCC tissues showed the significant activation and infiltration of CD8+ T cells into tumor masses in patients who had CD107a+ fractions. To explore the character of tumor reactive CTLs in primary tumor tissues, we analyzed the transcriptome of primary PD-1+CD8+ T cells, of which TCR genes (CDR3) were matched with tumor reactive CTLs. Omics analysis of tumor reactive population in primary tumor revealed that tumor reactive CTLs had the expression of HSP family and S100A family.

Conclusion In our study, we successfully isolate autologous tumor reactive CTLs from patient primary tumor in vitro. These CTLs may be available for T cell therapy against tumor in Liver, Pancreas, and Biliary tract. Now, we are going to do further experiments to characterize autologous tumor reactive population.

Keywords: T cell therapy, Personalized immunotherapy, Hepatocella Carcinoma, Single cell analysis.

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## A027 / Engineering CAR NK cells for antigen-dependent expansion

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A major limitation of CAR.NK cells therapy is the limited expansion capacity following target cell recognition relative to CAR-T cells. In mice, expansion and memory response of NK cells to MCMV was shown to be mediated by receptor activation as well as IL-12 signaling, indicating that both signals are essential for NK

cell expansion and the initiation of NK cell memory formation. However, in most cancers, the secretion of IL-12 by myeloid cells is null. To translate the NK cells expansion and memory response from anti-viral to anti-cancer therapy, we designed a chimeric cytokine receptor (CCR) system based on the integration of the GM-CSF receptor and the IL-12 receptor. Consequently, cytokine secretion following NK cell activation will result in autocrine IL-12 signaling, therefore supporting modularly NK cell expansion and initiation of anti-tumor NK cell memory response specifically at the tumor site.

Keywords: Natural Killer, Expansion, Chimeric antigen receptor, Chimeric cytokine receptor.

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## A028 / Identification of mutant IDH1 specific T-cell receptors from a glioma vaccine trial and a humanized mouse model

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Central to clinically relevant immunotherapy of gliomas is the discovery of tumor-specific antigens and the development of therapeutic strategies that effectively exploit them. One such example is the mutation in the gene encoding isocitrate dehydrogenase 1 (IDH1). This tumor-specific mutation is found in greater than 70% grade II and grade III gliomas. We have previously shown the therapeutic efficacy of a peptide vaccine encoding the IDH1R132H (mIDH1) in an MHC-humanized A2.DR1 mouse tumor model. In turn, the immunogenicity of the peptide vaccine was successfully tested in a multicentre, first-in-man clinical trial (NOA-16). The effectiveness of peptide vaccines is, however, limited by the ability of a patient to mount an immune response. This can be circumvented by employing adoptive cell therapy using T-cells transgenic for T-cell receptors (TCRs) against tumor-specific antigens. To identify mIDH1 specific TCRs, we first subjected patient PBMCs post-vaccination to antigen-specific in vitro T-cell expansion to enrich relevant T-cell clones. Candidate TCRs were identified using single-cell VDJ sequencing of expanded PBMCs and were then delivered into target cells using a safe, novel S/MAR based gene therapy vector. The functional validity of these TCRs was probed using NFAT reporter based T-cell activation assay optimized for testing TCRs against antigens presented on MHC-II complexes. In turn, we developed a protocol for rapid expansion of patient PBMCs to facilitate testing of TCRs in an autologous, HLA-agnostic setting when patient sample is limited. A similar approach was used to identify TCRs from an antigen-specific T-cell line generated from vaccinated A2.DR1 mice. We are currently generating TCR-retrogenic mice to test the capacity of these TCRs in an adoptive T-cell therapy setting to recognize and eradicate mIDH1 positive tumors. The proof of principle identification of mIDH1 reactive TCRs demonstrates the feasibility of exploiting immune responses against neo-epitopes as a first step in developing an adoptive TCR-transgenic T-cell therapy for glioma patients.

Keywords: T-cell receptor, IDH1, glioma.

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## A029 / Epstein-Barr Virus-induced CD137 expression in nasopharyngeal carcinoma

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Epstein-Barr virus (EBV) is an oncogenic virus associated with the pathogenesis of cancers such as Hodgkin's lymphoma (HL) and nasopharyngeal carcinoma (NPC) (Küppers et al., 2012; Tsao et al., 2015). EBV proteins and microRNAs have been identified to modulate the host immune environment to establish latency and evade immune defense (Chou et al., 2008; Cosmopoulos et al., 2009; Zheng et al., 2007). Latent membrane protein 1 (LMP1), an EBV protein, has been shown to transform B cells to adopt properties akin to those of malignant cells of HL (Vockerodt et al., 2008). LMP1 can also upregulate CD137 expression in T and NK cell lymphomas (Yoshimori et al., 2014). CD137 is a co-stimulatory molecule expressed by activated T cells but CD137 is also ectopically expressed by cancer cells in HL and helps to evade the immune response (Ho et al., 2013; Shao et al., 2015). The interesting link between EBV and ectopic CD137 expression in HL led to the hypothesis that NPC cells may have EBV-induced CD137 expression to facilitate immune suppression. This hypothesis was supported by CD137-positive staining in 42/122 (34%) cases of NPC patient tissue by immunohistochemistry (IHC). The subset of cells expressing CD137 was further identified to be NPC cells (77/116, 66%) by multiplex IHC staining for cytokeratin (epithelial cells), EBV encoded small RNAs (EBERs) and CD137. LMP1 was transiently transfected into NPC cell lines and was observed to induce CD137 expression. Stable NPC cell lines expressing CD137 were established to study the function of CD137 in NPC. CD137-expressing NPC cells were co-cultured with CD137 ligand (CD137L)-expressing B cell lines to show CD137-CD137L interaction and CD137L downregulation by trogocytosis. The role played by CD137 in the NPC stroma is currently being investigated in vitro.

Keywords: Nasopharyngeal carcinoma, Epstein-Barr virus, CD137.

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## A030 / Pre-clinical evaluation of an NY-ESO-1-specific TCR candidate generated using AgenTus' proprietary mammalian Retrocyte Display-based discovery platform

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AgenTus Therapeutics has developed a unique TCR discovery platform (T-RX) based on the mammalian Retrocyte Display functional screening system. T-RX is a differentiating platform that allows screening of large, fully-human TCR libraries in a high-throughput format and enables identification of TCRs based on both binding to the antigen/MHC of interest and functional signaling in T cells. We hypothesized that high affinity TCRs identified using this platform will confer potent anti tumor responses when delivered into autologous CD4 and CD8 T cells isolated from patients with advanced stages of cancer. We chose to target NY-ESO-1 since clinical responses in solid tumors have been observed in several adoptive T cell therapy clinical trials targeting

this antigen without severe toxicities. NY-ESO-1 is a prototypic cancer-testis antigen which is expressed by cancer cells and absent in normal somatic cells. Herein, we present preclinical studies performed with our lead TCR candidate and demonstrate the expected safety and feasibility of this approach. For lead candidate selection, we used an in vitro-transcribed (IVT) mRNA-based approach to express TCRs in Jurkat cells and primary T cells. One lead NY-ESO-1:A*02:01 candidate molecule was selected for subsequent optimization using the T-RXTM platform. Following CDR3 mutagenesis and guided selection (TCR α/β shuffling), 20 additional candidates with improved activity were identified, from which 3 were selected for further characterization. Human T cells collected from HLA-A*02:01 positive healthy donors were activated and transduced with lentiviral vectors encoding the 3 lead TCR candidates or a reference TCR as positive control. The majority (50-90%) of T cells transduced with AgenTus TCRs demonstrated NY-ESO-1:HLA-A*02:01 tetramer binding. Co-culture of AgenTus TCR T cells with NY-ESO-1(+) tumor cells showed target-dependent upregulation of activation markers: CD69, CD25, 4-1BB and OX-40. Furthermore, when interrogated for in vitro cytotoxicity, a cell dose-dependent and target-specific lysis of NY-ESO-1(+) tumor cells was observed for all 3 AgenTus TCRs; with no response observed with non-transduced T cells. This was corroborated by analysis of cytokine secretion, indicating polyfunctional immune responses. The potency of AgenTus TCRs was investigated in vivo using a xenograft model in immune-deficient mice, established by implantation of the K562 cell line overexpressing HLA-A*02:01 and NY-ESO-1. Treatment of tumor-bearing mice with engineered T cells led to significant regression in tumor volume and extended survival. Importantly, the strongest anti-tumor responses were observed with the lead candidate TCR, with complete tumor eradication observed post one week following T cell injection. Efficacy was comparable to treatment with the reference TCR-bearing T cells. Based on these findings, along with optimal cytotoxicity and cytokine secretion, we selected a development candidate that is now designated agenT-842, our clinical drug product. agenT-842 displayed a robust specificity profile when tested against single amino acid substitutions of the NY-ESO-1 peptide. The candidate is on track for IND/CTA filing in Q42019.

In conclusion, we believe that agenT-842, discovered using our proprietary T-RX platform, will be broadly applicable to tumors expressing NY-ESO-1 with high impact anti-tumor activity expected in solid tumors as a monotherapy with expanded clinical activity in planned combinations with our proprietary checkpoint modulating antibodies developed at Agenus, Inc. Moreover, this discovery platform is also applicable to multiple target types, including non-traditional neo-antigens that are shared within and between indications.

Keywords: Adoptive Cell Therapy, TCR, NY-ESO-1.

## A031 / "Switchable" antibody via reversible and responsive chemical modification for safe and efficient cancer immunotherapy

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Cancer immunotherapy, such as checkpoint blockade antibody therapy, has achieved remarkable clinical success leading to durable response in some patients with advanced-stage cancers. However, major challenges remain to benefit the majority of cancer patients with immunotherapy. Combination therapies have shown the potential to further increased the efficacy but could be limited in clinical applications due to greatly increased toxicities concurrently. For example, when anti-CTLA-4 was combined with anti-PD-1 antibody, enhanced anti-tumor activity came at the cost of synergistically exacerbated toxicity; ~55% of previously untreated melanoma patients given the combination experienced grade 3 or 4 adverse events. In addition to checkpoint blockade antibodies, antibodies against costimulatory molecules such as 4-1BB, OX40, and CD28, represent another class of promising cancer immunotherapies but has shown limited clinical use so far due to their sever systemic toxicities. Therefore, new technologies are highly desired to harness the potential of antibody-based immunotherapies of high potency while avoiding the concurrent toxicities. Here, we developed a "switchable" technology based on facile and reversible chemical modification of antibodies for safe and efficient cancer immunotherapy. The switchable antibody (Sw-Ab) was fabricated by masking native Ab with biocompatible hydrophilic polymers through the formation of reversible covalent bonds that responded to specific physiological triggers in tumor microenvironment. The Sw-Ab remained "off" status in circulation or healthy tissues, but turned "on" in tumor tissues via releasing the polymer masks in response to the tumor-specific triggers, such as low pH, high reducing potential, ROS, etc. To proof the concept, we have synthesized a pH-responsive Sw-Ab through conjugating poly(ethylene glycol) (PEG) onto a costimulatory monoclonal antibody, anti-4-1BB, through an acid-labile methylmaleic anhydride linker. The successful PEG conjugation was verified by dramatic increased molecular weight post modification evidenced by SDS-PAGE and HPLC analyses. The complete removal of masking polymers was validated by SDS-PAGE and HPLC analyses upon incubating the Sw-Abs in acidic buffer (pH = 6.5, mimicking tumor microenvironment) at 37 ºC overnight. We assessed the masking and recovery of anti-4-1BB activity through an in vitro T-cell binding assay. The Sw-anti-4-1BB Ab at "off" status exhibited 25 folds higher EC50 value comparing with native anti-4-1BB, while almost fully recovered at "on" status. Importantly, circulation half-life and anti-tumor efficacy of Sw-anti-4-1BB Ab remained similar as the native anti-4-1BB Ab in in vivo studies with C57BL/6 mice bearing B16F10 melanoma. Notably, Sw-anti-4-1BB Ab treatment exhibited minimal toxicities in the treated mice while mice treated with the same dose of native anti-4-1BB Ab showed splenomegaly and increased NK cell depletion in spleen and liver. Together, the Sw-Ab technique improved the safety of immunotherapy while maintaining its potency. We have also shown that the Sw-Ab technique could be extended to many immune-stimulating antibodies including checkpoint blockade antibodies (anti-PD-1 and anti-CTLA-4) and costimulatory antibodies (anti-CD40) and is a generalized strategy for next generation antibody-based immunotherapy.

Keywords: antibody-based immunotherapy, "switchable" antibody, reversible chemistry.

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## A032 / Graphene oxide modulates dendritic cell ability to promote T cell activation and cytokine production

Helen Parker (University of Manchester).

An important aspect of cancer immunotherapy is the ability of dendritic cells (DCs) to prime T cell immunity, an approach that has yielded promising results in some early phase clinical trials. However, since tumor immunogenicity is often weak, novel approaches are required to improve DC immunotherapy efficacy by enhancing their uptake of, and activation by, cancer antigens. Graphene oxide (GO) may provide a unique way to deliver antigen to DCs and modulate their ability to initiate effective adaptive immune responses. We have assessed whether GO affects DC activation and function, including their ability to take up, process and present the well-defined model antigen ovalbumin (OVA). We have found that GO flakes are internalised by DCs, while having minimal effect on their viability, activation phenotype or cytokine production. Although adsorption of OVA protein to either small or large GO flakes promoted antigen uptake into DCs, large GO interfered with OVA processing. In terms of modulation of DC function, delivery of OVA via small GO flakes enhanced DC ability to induce proliferation of OVA-specific CD4+ T cells, promoting granzyme B secretion. On the other hand, delivery of OVA via large GO flakes augmented DC ability to induce proliferation of OVA-specific CD8+ T cells, and their production of IFN-gamma and granzyme B. Together, these data demonstrate the capacity of GO of different lateral dimensions to act as a promising antigen delivery platform for DC modulation of distinct facets of the adaptive immune response, information that could be exploited for future development of targeted cancer immunotherapies.

Keywords: Dendritic cells, Antigen, Graphene oxide.

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## A034 / Immunogenic lipid fractions and infiltrating unconventional T-cells in pediatric papillary renal cell carcinoma

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Papillary renal cell carcinomas (RCC) rarely occur in children, which is why information about tumor biology and therapy is very limited. So far, the treatment of choice for pediatric papillary RCC is a complete surgical resection without any adjuvant therapy. Due to the rarity of the disease and the good survival, no further therapy for children with papillary RCC is defined. Nevertheless, it is important to further characterize this pediatric tumor entity and to expand the treatment options for the difficult cases. Beside the standard therapeutic options like surgical resection, chemotherapy and radiation, immunotherapy is emerging as an important therapeutic tool for childhood cancer entities. It is known, that tumor cells can be recognized and killed by conventional and unconventional T cells such as invariant natural killer T cells (iNKT cells) and γδ T cells. Unlike conventional T cells, antigen recognition is independent of major histocompatibility complex (MHC) molecules in iNKT and γδ T cells and this offers new alternatives for cancer immunotherapies. Vδ1 positive cells, a subgroup of γδ T cells, and iNKT recognize lipids via CD1d molecules. The nature of those lipids is largely unknown and α-Galactosylceramide is currently used as a synthetic model antigen. Here, we aimed to identify infiltrating unconventional and conventional T cells and the potential immunogenic lipids in pediatric papillary RCC. By flow cytometric analysis of isolated tumor infiltrating lymphocytes (TILs) of two pediatric papillary RCC samples, we detected the infiltration of conventional T cells, iNKT and γδ T cells. The infiltration of Vδ1 T cells could be identified in one of two samples. The infiltration of CD45+ and γδ T cells was confirmed by immunohistochemistry in both samples. CD1d expression was detected by qRT-PCR and IHC in both samples. To identify immunogenic lipids, we fractionated hydrophobic and polar tumor lipids and identified fractions able to induce the proliferation of iNKT and Vδ1 cells of healthy donors. Importantly, one hydrophobic lipid fraction was able to induce the proliferation of iNKT cells isolated from PBMCs and from TILs of one of the two papillary RCC patients and one polar fraction was able to induce the proliferation of Vδ1 cells isolated from the PBMCs of the same patient. In summary, pediatric papillary RCC are infiltrated by conventional and unconventional T cells and express lipids that can stimulate the proliferation of unconventional T cells. Our project is expected to provide new insights into the therapeutic utility of unconventional T cell therapy and it helps to get new information about the tumor immunology of pediatric papillary RCCs.

Keywords: Lipid Antigens, Unconventional T-cells, Pediatric papillary RCC.

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## A035 / Adoptive cell transfer following personalized cancer vaccination elicits newly detectable neoantigen-specific T cell responses endowed with anti-tumor activity in ovarian cancer patients

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Private tumor neoantigens derived from non-synonymous somatic point mutations can be immunogenic and are becoming highly attractive targets for tailored mutanome-based immunotherapies. A pilot study conducted by Kandalaft and colleagues has shown that an autologous oxidized tumor lysate-pulsed dendritic cell vaccine in advanced ovarian cancer patients is feasible and safe, and induces a broad antitumor response including T cell reactivities against private mutated neoepitopes. Patients who developed antitumor T cell responses following vaccination but failed to achieve a complete remission (n=12), were enrolled in a second part of the trial involving additional vaccine doses and lympho-depletion followed by adoptive cell transfer (ACT) of autologous vaccine-primed ex vivo co-stimulated T cells, as second line treatment. We performed a longitudinal screening of circulating neoepitope-specific CD8+ T cells by IFN-γ ELISpot in vaccinated patients who received T cell infusion (i.e. ACT patients). The TCRβ of CD137-sorted neoantigen-specific CD8+ T cells were sequenced and their frequencies analyzed in the T cell infusion product, where available, as well as tracked at different timepoints throughout therapy. Immuno-phenotyping and T cell fitness were assessed by flow cytometry over-time. Moreover, circulating tumor DNA (ctDNA) mutation profiling by high-throughput sequencing was performed on longitudinal serum samples. Circulating neoepitope-specific CD8+ T cells were identified in 7 out of 12 ACT patients. Of interest, several (n=7) CD8+ T cell reactivities to neoepitopes were newly detected only post-ACT in the blood of 5 out of 7 patients (ACT responders). Pre-ACT neoepitope-specific T cell responses were also amplified post-ACT. Neoepitope-specific T cells were oligoclonal and dominant TCRβ CDR3 sequences were identified. In ACT responders, CD8+ T cells preferentially expanded post-ACT while the circulating regulatory CD4+ T cell compartment decreased over-time as opposed to ACT non-responders (p=0.04). Of note, ctDNA encoding immunogenic, as opposed to non-immunogenic, mutations were preferentially detected and their levels specifically increased post-ACT (p=0.02), coincidentally with the detection of cognate mutation-specific T cell responses. Finally, ctDNA encoding immunogenic mutations was preferentially detected in patients clinically with stable disease (p=0.01). Taken together, our data show that ACT in the course of personalized vaccination induced several de novo circulating neoantigen reactivities, and amplified pre-existing responses to some neoepitopes. While the newly detected neoepitope-specific T cells were undetectable in the ACT product, a higher effector/Treg ratio was observed in ACT-responders post-treatment, suggesting that these patients were immunologically fitter for vaccination. Finally, the detection of ctDNA of immunogenic mutations suggests a role for the treatment-induced neoantigen-specific T cells in tumor control. These findings will have important implication for the immune-monitoring of T-cell responses, following immunotherapy, but also for developing new ACT strategies targeting neoepitopes in combination with personalized cancer vaccination.

Keywords: Adoptive cell transfer, Neoantigen-specific T cells, Ovarian cancer.

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## A036 / Abrogation of HLA surface expression using CRISPR/Cas9 genome editing to generate universal donor T cells

Munkyung Kim (Mogam institute for biomedical research), Jeewon Lee (Mogam institute for biomedical research), Joong-Hyuk Sheen (Mogam institute for biomedical research), Jihye Ryu (Mogam institute for biomedical research), Yu Young Kim (Mogam institute for biomedical research), Okjae Lim (Mogam institute for biomedical research).

Immune cell therapy including chimeric antigen receptor (CAR) T cells has revolutionized the way cancers are treated. Furthermore, the allogeneic donor-derived CAR T cells can pave a way to the off-the-shelf CAR-T cell therapy. However, rejection of the adoptively transferred allogeneic T cells caused by the host immune system remains an obstacle for the universal CAR T cell therapy. To investigate the role of HLA molecules in rejection of donor T cells, we attempted to inhibit expression of HLA molecules through the CRISPR/Cas9 gene editing system. First, we screened 60 gRNAs targeting B2M and 60 each gRNA targeting alpha chain of HLA-II molecules (DP, DQ and DR, respectively), and identified gRNA sequences highly efficient in target deletion without carrying off-target effect. By simultaneous quadruple genome editing of primary human T cells, we documented that HLA-I/II double negative T cells are phenotypically unaltered and functionally intact compared to non-target gRNA treated control cells. We next co-cultured HLA-I or HLA-I/II negative donor T cells with allogeneic responder peripheral blood mononuclear cells (PBMC) and analyzed alloresponses from responder. The overnight mixed lymphocyte reaction assays demonstrated that HLA-I negative T cells resulted in decreased IFN-γ & TNF-α production in the responder cells while deficiency of HLA-I/II in T cells further dampened alloresponses, positioning HLA-II as a key intermediary of alloresponse during allogeneic cell therapy. In summary, our approach will provide an invaluable tool to target HLA expression for the universal donor cell production.

Keywords: cell therapy, HLA, CRISPR/Cas9, universal donor T cells.

## A039 / First direct immunotherapy against HLA-G : anti-HLA-G CAR T cells

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The aim of this work was to develop a new immunotherapeutic strategy against the tumor associated antigen (TAA) and immune checkpoint (ICP) HLA-G to (i) overcome its immune-tolerogenic functions involved in tumor immune escape, (ii) to target HLA-G as a TAA and (iii) prevent suppressive microenvironment related to HLA-G. Chimeric antigen receptor T (CAR-T) cells therapies are demonstrated to be very efficient against hematological malignancies. However, it could not be applied to solid tumors since specific antigens are missing. The non-classical MHC class I HLA-G was identified as an excellent tumor specific antigen for both hematological and solid tumor cells. HLA-G expression is (i) associated with malignant transformation, (ii) is found on tumor cells and (iii) is rarely observed in healthy tissues defining HLA-G as a remarkable tumor associated antigen (TAA). Furthermore, HLA-G was recently identified as an ICP molecule exerting its tolerogenic functions through LILRB1 and B2 receptors binding, inhibiting all immune cell subsets and inducing regulatory T cells and suppressive APCs. Indeed, contrary to PD-1 / PD-L1, HLA-G presents restricted expression but wide immune-inhibitory functions. Despite several approaches aimed at targeting HLA-G, no immunotherapy against HLA-G-expressing tumor cells has been developed. Indeed, neither stimulatory functions nor cellular responses directed against allogenic HLA-G have been reported. We have generated new antibodies that are highly specific and bind strongly to immunosuppressive isoforms of HLA-G. VL/VH paratopes were used to develop 3rd generation CAR constructs to allow cytotoxic T lymphocytes redirection against HLA-G positive tumor cells. We first demonstrated that anti-HLA-G CAR-T cells generated were (i) specific and cytotoxic against tumors expressing HLA-G1 (K562-HLA-G1 and Jeg-3 cell line), (ii) bypassed the classical HLA-G/LILRB1 inhibition and were (iii) long-term effector memory CAR-T cells that could dampen tumor suppressive microenvironment. Finally, anti-HLA-G CAR-T cells functions were confirmed to migrate and to lyse HLA-G expressing tumor cells in vivo demonstrating that anti-HLA-G CAR-T immunotherapy is strongly efficient to control tumor progression and eradicate HLA-G tumor cells. We show that HLA-G CAR expressing T cells can be redirected against HLA-G, develop a cytotoxic phenotype and specifically lyse K562-HLA-G1 and JEG-3 tumor cells. Transduction efficiencies were >50% and stable beyond one month. We report here for the first time specifically targeting HLA-G for immunotherapy.

Keywords: CAR-T, HLA-G, solid tumor, tumor microenvironment.

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CHIMERIC ANTIGEN RECEPTORS AGAINST HLA-G (Patent EP19305809)

## A040 / In-depth characterization of tumor-reactive T cells by highly multiplexed immunofluorescence imaging

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The adoptive cell transfer (ACT) of tumor-infiltrating lymphocytes (TILs) has shown remarkable results in patients with different cancer types. The antitumor effect of this therapy is mainly attributed to a small fraction of tumor-reactive T lymphocytes (TRLs) that recognize mutated peptides (neoantigens) as well as (overexpressed) self-antigens. Therefore, the enrichment and expansion of TRLs constitutes a promising immunotherapy approach. However, the specific targeting of individual mutated antigens in a patient´s cancer presents a daunting challenge for widespread therapeutic application. Alternatively, we hypothesize that TRLs could be identified by a surface marker (or combination thereof) in an antigen-independent manner as a result of the chronic antigen exposure and other factors present in the tumor microenvironment (TME). We thus aim to phenotypically characterize the TIL compartment and, more precisely, TRLs to improve and facilitate ACT approaches. For this purpose, we screened T cell activation and exhaustion markers, among others, on different tumor types sections using the MACSima™ Imaging Platform, an instrument for the highly multiplexed immunofluorescence imaging technology MICS (Multiparameter Imaging Cell Screen), enabling investigation of hundreds of markers simultaneously while preserving the spatial information. Image segmentation and bioinformatic tools were used to analyze and integrate the large amount of data generated. Moreover, flow cytometry and immunoprofiling of T cell repertoires coupled with single cell RNA sequencing were performed to complement the characterization of TILs. Here we demonstrate the technology for epithelial ovarian cancer. The MICS results highlighted the complexity of the TME, composed by tumor cells, fibroblasts and endothelial vessels, as well as an extensive infiltrate composed of different immune cells, such as T cells, plasma cells and distinct myeloid cells. Particularly, T cells from different tumor areas exhibited a tissue-resident memory phenotype with the expression of CD69, CD45RO or

CD103, as well as an activated/exhausted state determined by the upregulation of different markers such as PD-1, TIM-3 or CD39. Furthermore, different T cell subsets were identified based on their phenotype, and the interaction with other cell types (e.g. immune cells, tumor cells) was studied by calculating proximities between cell groups. Flow cytometry data revealed similar T cell phenotypes. By sequencing T cells on single-cell level dominant TCR clones were found, indicating an ongoing clonal expansion in the tumor. Moreover, RNA expression patterns were examined and correlated to the protein expression profile obtained by MICS.

In conclusion, this novel imaging technology offers the possibility to study multiple parameters—including the localization—of relevant cells in the TME such as T cells. The phenotypic and functional characterization of different T cell subsets will allow the further investigation of their potential anti-tumor reactivity. Ultimately, the enrichment and expansion of the identified tumor-reactive T cell population hold great promises to improve the efficiency of T cell therapy against cancer.

Keywords: Tumor-reactive T cells, Multiplexed Imaging, Ovarian cancer.

## A041 / Personalized dendritic cell-whole tumor lysate vaccination synergized with immunomodulatory therapies to enhance neoantigen T cell responses in ovarian cancer

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Hypochlorous acid-oxidized whole tumor lysate-dendritic cell (OCDC) vaccination was feasible in recurrent ovarian cancer (OC). We successfully elicited polyclonal T cell responses against different tumor-associated antigens in immunotherapy-naïve OC subjects. OCDC vaccination also induced “de novo” T cell responses against previously unrecognized private tumor neoantigens, as well as priming significantly higher avidity (~100-fold increase) T cells against previously recognized neoepitopes. Overall, the elicited tumor-specific T cell responses was associated with significantly prolonged overall survival in the treated OC subjects. When we combined OCDC vaccination with bevacizumab and metronomic cyclophosphamide, we observed a significant prolongation of overall survival and enhanced anti-tumor immune responses in the OC subjects. Bevacizumab and metronomic cyclophosphamide were used to target the tumor vasculature and T regulatory cells, respectively, in the OC tumor microenvironment. They also synergized with OCDC to improve the infiltration of T cells into the tumors. In this current study, we combined the previous OCDC vaccination regimen with aspirin and IL-2 to further improve the efficacy in OC subjects. Aspirin was shown to inhibit cyclooxygenase 1 (COX1) and COX2 activity and prostaglandin 2 production that subsequently improved T cell homing to the tumors. IL-2 served as a T cell growth signal that further amplified the anti-tumor T cell responses induced by OCDC vaccination. To evaluate the molecular and cellular immune mechanisms involved, we used an established ID8 murine OC tumor model and demonstrated that OCDC vaccination indeed elicited neoantigen-specific T cells. These neoantigen-specific T cells secreted IFN-gamma in recognition of ID8 tumor cells ex vivo. We also observed that the addition of anti-VEGF, cyclophosphamide, aspirin and IL-2 to OCDC vaccination significantly reduced tumor burden (P-value = 0.0008) and prolonged overall survival (P-value = 0.009) in the ID8-tumor bearing mice. Similarly, we observed prolonged progression-free survival till expiration in OC subjects who were treated with the same treatment strategy. We are currently evaluating the elicited neoantigen-specific T cells for their cytolytic function and cytokine production, as well as identifying the immune signature associated with this treatment strategy. These results will assist in future planning to efficacious treatment combinations in OC.

Keywords: Hypochlorous acid, Dendritic cells, Neoantigen-specific T cells, Anti-tumor responses.

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## A042 / CAR-T therapy for prostate cancer

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Prostate cancer (PCa) affects one in six men. Treatment options for men with localized disease include radical prostatectomy or brachytherapy. Unfortunately, patients may also present with advanced disease, which is lethal due to treatment resistance. There is an unmet demand to develop new therapies that improve outcome. Harnessing the immune system offers great potential in this regard. In this study, we generated second-generation Chimeric Antigen Receptor T cells targeting a carbohydrate antigen, Lewis Y (LeY-CAR T), with a truncated CD34 surface molecule for purification. Lewis Y is highly expressed in PCa, particularly in higher grade primary PCa and metastatic disease. By using FACS and CyTOF analysis, we found that the CAR T product typically generated in our clinical protocols consists predominantly of well differentiated effector cells and effector memory cells. Evidence is now emerging that the differentiation state of the CAR T product can have an impact on the therapeutic response and post-infusion persistence. Therefore, we optimized the manufacturing protocol. Briefly, PBMC was activated by CD3/CD28 soluble tetrameric antibody complex and the cells were transduced twice with retrovirus carrying LeY-CAR construct. LeY-CAR T cells expanded with optimal dose of IL-7/IL-15 exhibited predominant earlier differentiation phenotypes, including stem cell-like memory phenotype and central memory phenotype, defined by CD95+CD62L+CCR7+. Using the optimized protocol, we produced CAR T cells with 20- to 30-fold expansion and over 98% purity in 6 to 10 days. We demonstrated that LeY-CAR T cells retained potent antigen-specific cytotoxicity and the capability to secrete cytokines such as TNF- $\alpha$ and IFN-$\gamma$ in vitro. Our in vivo studies also showed that infused CAR T cells can reduce tumor burden and persist post infusion. In summary, we produced CAR T cells with high purity and early memory phenotype in a shortened timeframe. This may further benefit the clinical application of CAR T for PCa, by enriching the infused product with T cell that have specificity for LeY and improving anti-tumor efficacy and persistence in vivo.

Keywords: Prostate Cancer, CAR T, Lewis Y.

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[1] Reducing Ex Vivo Culture Improves the Antileukemic Activity of Chimeric Antigen Receptor (CAR) T Cells, Saba Ghassemi, Michael C Milone, et al, Cancer Immunol Res August 22 (2018)

## A043 / An HLA-agnostic and functional approach to identifying and engineering TCR specificity against solid tumor neoantigens

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Neoantigens (NeoAg) offer attractive therapeutic targets for directing a patient's immune response to the immunogenic subset of mutations expressed exclusively by cancer cells. Despite the specificity with which NeoAg enable tumor recognition, the majority of identification approaches rely purely on predictive Methods such as peptide-HLA binding affinity. These methodologies have met with limited success in revealing natural targets present on tumor cells. We have developed a novel, HLA-agnostic, and functional approach to NeoAg identification which combines genomic sequencing with bioinformatic analysis. We nominate mutations for subsequent functional analysis using patients' T cells by identifying natural responses generated under physiologic conditions. Exploiting this, we identified a missense mutation (V205I) in the ribosomal protein RPS2 that is recognized by CD8+ T cells from tumor-infiltrating lymphocytes (TIL) of a metastatic HPV-16+ Head and Neck Squamous Cell Carcinoma lesion. Importantly, this nearly monoclonal CD8 response is directed solely against the NeoAg (RPS2 V205I) and not HPV transforming proteins. Next, we used single-cell transcriptomics to isolate the genes encoding the RPS2-specific TCR and show that it recognizes a minimal, mutated peptide bound to HLA-B*07.02. Further, we established an inducible cancer stem cell line that retains RPS2 expression and the V205I mutation but lost HLA-B*07:02 expression. Reintroducing the restricting HLA allele results in tumor-specific recognition and killing by autologous TIL and TCR engineered allogeneic T cells both in vitro and in vivo. These results demonstrate that high-affinity NeoAg-specific T cell responses can be identified in cancer patients and that the relevant TCR can be isolated for use in TCR engineering-based adoptive cell therapy.

Keywords: Adoptive Cell Therapy, TCR engineering.

## A044 / Automated ex vivo isolation and expansion of CD137-positive tumor-reactive T cells on the CliniMACS Prodigy®

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Adoptive cell transfer (ACT) of tumor-infiltrating lymphocytes (TILs) have shown remarkable results in patients with metastatic melanoma. However, only a small fraction within the TIL population reacts against the tumor. Therefore, the pre-enrichment of tumor-specific T cells and subsequent ex vivo expansion may improve the efficiency of ACT therapies. In addition, tumor-reactive T lymphocytes circulating in the blood (TRLs) have been found in low frequencies, which represents a challenge for their isolation, but also an advantage over TIL therapy since blood is a more reliable and accessible source than tumor excisions. Another impediment to the widespread application of ACT is the conventional rapid expansion protocol (REP) that constitutes a laborious and extensive process with frequent culture manipulations, and thus requires specialized personal and equipment. Our aim is to develop a fully automated large scale ex vivo T cell isolation and expansion procedure in the CliniMACS Prodigy in order to simplify the manufacturing of tumor-reactive T cells for ACT. The CliniMACS Prodigy instrument is a controlled system that integrates a series of cell processes, from magnetic cell separation and cell culture to final product formulation, under GMP conditions in a closed system. We have developed and optimized an automated process that allows for the cultivation of single-cell suspensions of tumor digest or peptide-stimulated leukapheresis, followed by isolation of T cells expressing the activation marker CD137 using clinical grade reagents. Subsequently, the tubing set is cleaned and the positive fraction can be expanded in the CentriCult Unit following addition of MACS GMP CD3 pure, excess of irradiated feeders, and high doses of MACS GMP Recombinant Human IL-2. For the development, we used both cryopreserved tumor digests as well as virus-specific T cells from healthy donor leukapheresis and obtained high purities and optimal recovery of antigen-reactive CD137-positive T cells following stimulation (using viral Pep-Tivators against EBV, CMV and AdV in the case of healthy donor leukapheresis). The isolated fractions (as low as 2E4 up to 3E6 T cells) could then be expanded using the conditions mentioned above in an automated fashion in TexMACS GMP medium with 3% AB serum; optimal shaking and feeding strategies were defined as the process has an activity matrix that allows for high flexibility regarding culture parameters. Expansions ranged from ~1, 000 to 15, 000 fold, which mainly depended on the starting number of responding cells – in one extreme example an expansion of ~80, 000 fold was achieved when starting with as low as 2E4 responder cells. In summary, these data provide proof of concept for the isolation and expansion of antigen-reactive T cells from tumor digest or from peripheral blood in a closed, automated manner in the CliniMACS Prodigy, allowing for the desired efficiency, simplicity and automated production of ACT therapies against cancer.

Keywords: Tumor-reactive T cells, CD137 (4-1BB), CliniMACS Prodigy.

## A045 / Analysis of the paired TCRα- and β-V(D)J full length chains of single cell sequence from human naïve and antigen-experienced T cells

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The unbiased and paired characterization of T-cell receptor (TCR) alpha and beta chains is critical to understand the TCR repertoire and adaptive immunity. However, the direct cloning of the single human full length TCRα- and TCRβ-chains is very few reported. We present a new methodology enabling capture natively paired, full-length rearranged T-cell receptor (TCR) sequences at single cell level of human T cells. More than 50 percent paired TCRα- and β- chains were captured in 100 single naïve human CD8+ T cells using 5'SMART and 3' TCRαβ constant region nested gene specific primers (GSP). Furthermore, we sequenced the TCR of the specific T cells that were stimulated by representative tumor-associated antigens (TAA), virus-related antigens (VRA) and neoantigens. The highest proportion of TCR subtypes were extracted in the TCR abundance ranking by IMGT blast. Finally, we obtained the paired, full-length V(D)J TCR from Melanoma antigen Melan A epitope 27-35 that was TRAV(12-2)J(47), TRBV(7-6)J(2-1), and the TCR from antigen EBV LMP2-FLY that was TRAV(26-1)J(7), TRBV(27)J(2-1). These results suggest the method provides accurate identification of the paired, V(D)J rearrangements for each individual human naïve and antigen-experienced T cells. The information could be used by the construct of an engineered T-cell receptor (TCR) for cancer immunotherapy and infectious diseases.

Keywords: TCR-T, Single cell sequencing.

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## A046 / An ultraspecific monoclonal antibody recognises a novel marker on stem memory T cells and induce cell proliferation and differentiation in vitro and in vivo

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This report describes the generation of an ultra-specific anti-SSEA-4 mouse IgG3 antibody (FG2811mG3) and the discovery of the expression of SSEA-4 on 0.5 to 2% of human and mouse stem memory T cells (TSCMs). Transcriptomic analysis of CH2811hG1 (chimeric IgG1 version of FG2811) positive T cells confirmed that they had a similar profile to TSCMs. For the first time, we showed that stimulation of purified human T cells using CH2811hG1 induced CD4 and CD8 TSCM cell proliferation and differentiation. CH2811hG1 stimulated TSCM cells remained viable for more than 2 months in vitro in the absence of exogenous cytokines. Assessment of cytokine and chemokine secretions in CH-2811hG1 stimulated human T cell culture suggested that IL-7 and IL-21 were the self-maintaining cytokines of TSCM cells. The splenocytes harvested from C57/B6 mice immunized with FG2811mG1 remained viable in the culture for more than 3 weeks without any exogeneous cytokines. At day 23, phenotypic analysis of these viable splenocytes showed that 31% were TN/ TSCM (CD3+CD44-CD62L+), 32% were TCM (CD3+CD44+CD62L+) and 38% were TEM/ TEFF (CD3+CD44+CD62L-) cells. Only TEM and TEFF cells were detected in the unimmunized splenocyte culture. Mice receiving vaccines in combination with FG2811mG1 antibody showed increased T cell responses. FG2811/CH2811 is unique in isolating and inducing proliferation of putative TSCM cells, which addresses the ongoing debate regarding the methodology for inducing TSCM cells from naïve or activated T cells. We envision that CH-2811hG1 positive TSCM cells are potential candidates for genetic manipulation to express cancer specific T cell receptors (TCRs) or chimeric antigen receptors (CARs). In addition, increased cellular immunity induced in combination with vaccines suggests it may have potential as a therapeutic agent for the treatment of solid tumors.

Keywords: Stem memory T cells, ultraspecific antibody, adoptive t cell transfer, CART.

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## A047 / Liquidation of solid tumors by in vivo expanded CLDN6-CAR T cells

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Adoptive cell therapy based on genetically engineered T cells armed with chimeric antigen receptors (CARs) shows tremendous clinical success in patients with B-cell malignancies. However, only limited anti-tumoral activity was observed in multiple clinical trials upon CAR T-cell therapy in patients suffering from solid tumors. Two key hurdles of CAR T-cell therapy targeting non-hematological neoplasia are i) the lack of highly cancer selective targets providing challenges for safe treatment protocols and, ii) the insufficient expansion and long-term persistence of CAR T cells probably due to inefficient co-stimulation upon antigen-exposure in vivo. Especially the latter point displays a fundamental difference comparing CAR-based treatment of B cell derived hematological malignancies and solid tumors. We introduce here a CAR T-cell therapy against Claudin 6 (CLDN6), an oncofetal cell surface antigen expressed in embryonic stem cells during fetal development. The gene encoding CLDN6 is strictly silenced and not expressed in healthy adult tissues but it is re-activated in different cancers with a high medical need including ovarian, endometrial, testicular and lung cancers. Based on in vitro studies, we selected a 4-1BB containing second generation CAR that exhibited a highly specific and sensitive recognition and killing of CLDN6 positive cells. T cells equipped with CLDN6-CAR were able to repetitively clear tumor spheroids in a stress test scenario recently proven to be predictive for in vivo efficiency. Accordingly, therapeutic studies in immunocompromised mice showed that CLDN6-CAR T cells mediate a complete eradication of advanced human tumors. To further improve the activity we developed a novel approach for stimulation of CAR T cells in vivo by antigen-exposure on professional antigen presenting cells. To this end we used on liposomally formulated mRNA (RNA-Lipoplex, RNA-LPX) to systemically deliver the corresponding antigen to dendritic cells (DCs) in secondary lymphoid tissues. Repetitive RNA-LPX treatment can expand CAR T cells in vivo in a dose-dependent manner resulting in an improved persistence. Similarly, we demonstrated an expansion of CAR T cells recognizing other antigens proving the broad

applicability of the approach. Further studies demonstrated that stimulation of CAR T cells by RNA-LPX treatment accelerates anti-tumor activity and can restore anti-tumoral efficacy even at insufficient CAR T-cell doses. Quantitative analyses of CAR-T cells demonstrated that repetitive antigen-exposure on lymphoid DCs in vivo allows a controlled expansion of CAR T cells thereby supporting a safer clinical translation of novel CAR T-cell therapies. A clinical trial investigating CLDN6-CAR therapy in combination with RNA-LPX treatment is currently planned in patients with advanced CLDN6 positive human cancers.

Keywords: Chimeric antigen receptor, solid cancer, adoptive T cell therapy, RNA vaccination.

## A048 / Targeting metastatic solid tumors via adapter chimeric antigen (AdCAR)-engineered NK-92 cells

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The vast majority of cancer-related deaths are a consequence of metastasis. Patients with advanced metastatic disease are, with some exceptions, incurable by current treatment options. Although great advances have been made in combatting cancer, particularly at its early stages, surgical resection, chemotherapy and radiotherapy show only limited efficacy in the elimination of metastases. Despite unparalleled therapeutic potential of chimeric antigen receptor (CAR) T cells in treatment of refractory hematological malignancies, developing effective adoptive immunotherapies for solid cancer represents a much greater challenge. Major obstacles include the identification of antigens expressed on tumor cells that can be targeted safely and the design of optimal CARs that both redirect immune cells to recognize tumors and overcome the immunosuppressive local microenvironment that interferes with immune cell mediated tumor eradication.

We recently developed a universal adapter CAR (AdCAR) system. Utilizing adapter molecules, we split antigen recognition and CAR-driven immune cell activation. The system allows precise quantitative (on-/off) as well as qualitative (combination of target antigen) regulation of immune cell function. AdCARs are based on the unique properties of a novel scFv targeting a "neo"-epitope-like structure derived from the endogenous vitamin biotin, which can be linked to a monoclonal antibody (mAb).

We intended to combine the universal targeting and controllability of the AdCAR with the "off-the-shelf" properties of the continuously expandable NK cell line NK-92. The cells were transduced with AdCARs containing either CD28 or 4-1BB co-stimulatory plus CD3-ζ signaling domains. Interestingly, only CD28 containing AdCARs sufficiently mediated specific target cell lysis in the presence of biotinylated antibodies. Using single cell sorting, AdCAR NK-92 clones with the highest CAR expression were selected and demonstrated significantly improved target cell lysis. Importantly, lysis of cancer cells by AdCAR NK-92 is largely independent from activation of endogenously expressed NK receptors and the presence of their ligands on target cells but mainly mediated by AdCAR activation through binding to the respective tumor antigen. To test the universal applicability of the AdCAR system we tailored a standardized antigen platform for the characterization of various newly established cell lines of different metastatic tumor entities (colon carcinoma, mamma carcinoma, renal cell carcinoma and melanoma). Via flow cytometry we were able to identify a variety of promising target antigens. In the presence of the respective antigen, first and foremost biotinylated antibodies directed at CD146, CD276, and EGFR were capable of inducing significant NK-92-mediated lysis against the tumor cell lines after merely 2 hours, while sparing healthy tissue. Importantly, irradiation of NK cells, as required in all active clinical trials using NK-92, prior to testing had no observable effect on target cell lysis or chemokine receptor expression.

Since one prominent mechanism by which metastatic tumor cells evade immune surveillance is the secretion of transforming growth factor beta (TGFβ), a highly immunosuppressive cytokine, we conducted in vitro assays to test the influence of TGFβ on NK-92 cells. Surprisingly, AdCAR-modified NK-92 cells retained efficient CAR-mediated killing even in the presence of TGFβ concentrations exceeding those found in cancer patients.

In conclusion, we have generated a CAR-modified NK cell line, AdCAR NK-92, whose effector function can be tightly regulated and redirected against one or multiple antigens allowing tunable and universal targeting. Moreover, AdCAR NK-92 cells can be manufactured as an "off-the-shelf" standardized product improving the practicality of NK CAR therapy combined with the possibility of tailoring a specific antigen platform for patient-individualized treatment of metastatic solid tumors.

Keywords: Chimeric Antigen Receptor, NK-92, Solid Tumors.

## A049 / Characterization of tumor antigen specific T cell receptors derived from a cervical cancer patient with benefits after cellular immunotherapy

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Transfusion of tumor specific T cell receptor (TCR)-transgenic T cells, namely, TCR-T therapy, has shown promising efficacy against solid tumor in clinical trials. However, tumor associated antigen (TAA)-specific TCRs with ex vivo affinity maturation showed occasionally lethal on-target/off tumor or off-target toxicities in patients. Therefore to select TAA-specific TCRs with optimal affinity to achieve both efficient and specific tumor targeting is of great importance for TCR-T therapy.

Previously, a cervical cancer patient with bone metastasis was treated with Multiple-Antigens Stimulating Cellular Therapy (MASCT), a cell-based immunotherapy. After treatment the patient showed partially response and remained stable disease for 5 years. Moreover, significant and durable T cell immune responses against diverse tumor antigens were observed in patient's periphery blood. Such MASCT-benefited patients provide a good source of safe and effective TCRs. Firstly, we identified from this patient's peripheral blood mononuclear cells (PBMCs) hundreds of TCR alpha/beta clonotypes with potential specificities against tumor antigen RGS5 and HPV18 E7 through enrichment of IFN-gamma-secreting T cells upon peptide-stimulation followed by NGS TCR sequencing. Secondly, we utilized single cell TCR sequencing technology and found out 35 and 41 paired TCR alpha/beta clonotypes, which were specifically enriched after RGS5 and HPV18 E7 stimulation, respectively. Of those 6 and 6 paired TCRs for RGS5 and HPV18 E7, respectively, were further cloned and lentivirally transferred into the patient's PBMC for validation of their antigen specificity and HLA-restriction. All of tested TCRs could be successfully transduced with varied efficiency (4-65%). Especially, there were each 5 TCRs specially recognizing RGS5 and HPV18 E7. PBMC transduced with each specific TCR secreted significantly IFN-gamma and/or TNF-alpha in response to the corresponding antigens as determined by ELISA and flow cytometry assay. Of note, all of 10 specific TCRs were CD4 TCRs restricted to HLA-DP or HLA-DR. Tumor specific CD4 T cells have shown long-term anti-tumor efficacy in clinical studies, implying that CD4 specific TCRs own great potential for TCR-T therapy. Some of identified specific TCRs recognized tumor antigens in restriction of very common HLA allotypes in Chinese population, suggesting a large proportion of patients could be benefited.

In conclusion we successfully isolated CD4 TCRs specific for tumor antigen RGS5 or HPV18 E7 from a cervical cancer patient with good clinical outcome after cell-based immunotherapy. Several TCR pairs with defined specificities are being further investigated in terms of affinity, tumor recognition as well as cross-reactivity

for potential application in TCR-T therapy against solid tumor.

Keywords: CD4 tumor-specific TCR, TCR-T, solid tumor, single cell TCR sequencing.

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## A050 / Pre-clinical characterization of a T cell receptor recognizing CD20/HLA-A2 to evaluate therapeutic efficacy and map in vivo and in vitro off-target reactivities

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Despite the recent progress using CAR19T cell therapy, the majority of lymphoma patients are still in need of improved treatment options. Targeting tumor associated antigens with T cell receptors (TCR) offers an alternative approach. High-affinity TCRs specific for CD20 presented on allogeneic HLA-A2 (TCR 20-1 and 20-2) were previously identified and characterized by our group. We demonstrated that T cells, modified to express either of these TCRs, efficiently killed HLA-A2+ CD20+ cancer cell lines and primary lymphomas, while sparing cells from a large variety of tissues that lacked either CD20 or HLA-A2. In light of toxicities observed in previous adoptive TCR clinical trials, an in-depth analysis of potential off-target reactivities is required prior to clinical application. As no comprehensive strategy for in vitro or in vivo safety testing of therapeutic TCRs has been published, our goal was to establish a pipeline for such a screening strategy, using TCR 20-1. To this end, we first mapped the residues critical for TCR recognition using a peptide library in which every amino acid position in the cognate TCR target peptide was substituted with every possible alternative amino acid, one at a time. Next, using the ScanProsite algorithm, an in silico search was performed for all naturally occurring peptides in the human proteome that contain any combination of the alternative amino acids that led to TCR recognition. A second peptide library was established of these sequences and we could exclude TCR 20-1 reactivity to the majority of them. We next determined whether the peptides that did induce a TCR-mediated response were naturally processed and presented on HLA-A2. GFP-labelled RNA constructs encoding 30-mer amino acid sequences containing the candidate cross-reactive peptide in a central position were introduced into antigen-presenting cells. IFN-gamma production by T cells expressing the TCR 20-1 was assessed, leading to the exclusion of all but three targets. Final tests using a cell line panel naturally expressing these three potentially cross-reactive proteins are ongoing. To evaluate the in vivo efficacy and safety of TCR20-1 and TCR20-2 T cells, we applied a syngeneic HLA-A2-transgenic (tg) cancer model. In this HLA-A2tg model, immune recognition relies on human molecules (TCRs 20-1/20-2, HHD (chimeric HLA-A2 molecule) and the targeted CD20 epitope), while all cellular components (cancer cells, T cells and host) are of mouse origin. In contrast to conventional xenograft models, this system captures all the complex interactions between tumor and immune cells and thus models the therapeutic context more accurately. We modified the fibrosarcoma cell line MC703 to stably express high levels of the CD20/HHD target on the cell surface and transplanted the cells subcutaneously into HHDxRag1-/- mice. After tumors were established, animals were treated by injection of TCR modified T cells. Adoptive therapy with TCR modified T cells led to tumor rejection in 95% (TCR 20-1) and 65% (TCR 20-2) of the mice. The murine proteome expressed on HHD in healthy mouse tissues allowed screening for possible TCR cross-reactivities as a large part of the human and murine proteome is shared. This provides important information regarding safety of these TCRs in an adoptive TCR therapy setting. Pathological analysis of murine organs (brain, heart, kidney and liver) 10 weeks after TCR 20-1 T cell treatment did not reveal any off-target effects.

In conclusion, we present here a comprehensive and systematic strategy to analyze therapeutic efficacy and to identify potential off-target reactivities in vitro and in vivo which can be applied in pre-clinical risk-assessment algorithms for future therapeutic TCRs.

Keywords: Adoptive T cell therapy, T cell receptor, in vivo efficacy, off-target reactivity.

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## A051 / ECM-targeted CAR T-cell therapy targeting oncofetal EDB-fibronectin for solid tumors

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Cell therapy with T-cells expressing chimeric antigen receptors (CARs) present a promising approach to improve outcomes for patients with recurrent/refractory solid tumors. Both the lack of suitable antigens and that most CAR T-cells only target the malignant component of solid tumors have been identified as major roadblocks. To overcome these limitations, we propose to target oncofetal fibronectin (FN-EDB, EDB) that is present in the extracellular matrix (ECM) of solid tumors, and is secreted by tumor and endothelial cells. We constructed a 2nd generation EDB-CAR and generated human EDB-CAR T cells by standard retroviral transduction. EDB-CAR T-cells were specifically activated by recombinant EDB protein, and recognized and killed EBD+ human tumor cells (A549 [lung], LM7 [osteosarcoma], U87 [glioma]) in contrast to non-transduced (NT) T-cells. In vivo, 1x10^6 EDB-CAR T-cells had potent antitumor activity in subcutaneous and systemic tumor xenograft models resulting in a significant survival advantage in comParison to NT T-cells. Tumors treated with EDB-CAR T-cells revealed a significant decrease in CD31+ endothelial cells in comParison to tumors treated with NT or control CAR T-cells, indicating that EDB-CAR T-cells target the tumor vasculature. However, EDB-CAR T-cells had limited antitumor activity against U87 cells in which EDB was knocked out by CRIPSR-Cas9 gene-editing technology, highlighting that tumor associated EDB is critical for the observed potent antitumor activity. Since human and murine EDB are 100% homologous, we also performed toxicity studies with 1x10^7 EDB-CAR T-cells, a T-cell dose that was 10-fold higher than the T-cell dose used in our efficacy experiment, and observed no dose limiting toxicities. Thus, EDB-CAR T-cells have potent antitumor activity with an encouraging safety profile in preclinical models, warranting further active preclinical

development.

Keywords: CAR T cell therapy, Cell Therapy, Tumor Microenvironment.

## A052 / First-in-human CAR T for metastatic breast cancers targets the MUC1 transmembrane cleavage product MUC1*

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We developed a MUC1* targeting CAR T that has been approved for a 1st-in-human clinical trial for metastatic breast cancers at the Fred Hutchinson Cancer Research Center that is scheduled to open in Q3 2019.

huMNC2-CAR44 targets MUC1* (muk 1 star), which is a transmembrane cleavage product of MUC1. Unlike full-length MUC1, MUC1* is a growth factor receptor that drives tumor growth and metastasis. MUC1* is activated when onco-embryonic growth factor NME7AB dimerizes its truncated extra cellular domain. The binding site for NME7AB is masked in full-length MUC1, as is the binding site for the huMNC2 antibody. Thus, huMNC2 does not bind to full-length MUC1, which is expressed on healthy epithelium. All other attempts at a MUC1 targeted therapeutic have failed. Our experiments strongly argue that they failed because they targeted the tandem repeat domain of full-length MUC1, which is shed after cleavage. About 30% of breast cancer tissues no longer express detectable levels of surface expressed full-length MUC1, compared to only 5% that did not express huMNC2-reactive MUC1*. A CAR T that targets full-length MUC1 would at best be ineffective; at worst, it would increase tumor growth by eliminating cells expressing the quiescent full-length MUC1 and enriching for the virulent MUC1* expressing population.

huMNC2-CAR44 T cells are highly selective for cancerous tissues because the huMNC2 antibody recognizes MUC1* but not the healthy full-length form. Additionally, huMNC2 recognizes a conformational epitope that is created when MUC1 is cleaved by an enzyme that is overexpressed in breast cancers and is an indicator of poor prognosis. In the absence of huMNC2-CAR44 treatment, injection of this cleavage enzyme near breast tumors in mice caused a dramatic increase in tumor volume over just a few days, requiring immediate sacrifice. IHC studies of thousands of normal vs. cancerous human tissue specimens showed that huMNC2-scFv almost exclusively binds to tumor tissues, hitting over 90% of breast, 83% ovarian, 78% pancreatic and 71% of lung cancers. Recognition of breast cancer specimens appears not to be limited by cancer sub-type. In vivo experiments of human tumors in NSG mice (n>300), show that huMNC2-CAR44 T cells robustly inhibited MUC1* positive solid tumors. A single injection of huMNC2-CAR44 T cells eliminated tumors in NSG mice which remained tumor free to Day 100, compared to control animals that had to be sacrificed at Day 20 due to excess tumor volume. Other animal experiments showed that huMNC2-CAR44 T cell efficacy increased with increasing antigen density. Dual tumor experiments of a high antigen density and low antigen density tumor in the same animal showed that adequate MUC1* density is required for a CAR T response, further supporting the idea that huMNC2-CAR44 T cells will selectively kill MUC1* positive tumors, while sparing normal tissues.

The generation of an anti-MUC1* CAR that recognizes a conformational epitope created when MUC1 is cleaved to MUC1* by a specific cleavage enzyme that is overexpressed by the tumor, argues that CARs could be made to be cancer sub-type specific, or even patient specific, based on which cleavage enzymes their tumors express.

Keywords: CAR T, breast cancer, MUC1.

## A053 / Persistent TCRB repertoire in blood found at vaccination site and tumor infiltrating lymphocytes in a cutaneous melanoma patient immunized with the CSF-470 vaccine plus BCG and rhGM-CSF

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The CASVAC-0401 study analyzed the combination of the CSF-470 cell-based vaccine plus BCG and rhGM-CSF for immunotherapy adjuvancy in stage II-III cutaneous melanoma patients after surgery, in comParison to medium dose IFN-a2b. Patient-045 developed a mature vaccination site (VAC-SITE) and a regional cutaneous metastasis (C-MTS) which were excised during the protocol. CDR3-TCRβ repertoire sequencing in PBMC and tissue samples, along with skin-DTH score and IFN-G ELISPOT assay in PBMC obtained before, during and at the end of vaccination were performed to analyze the T-cell immune response dynamics throughout the immunization protocol. Histopathological analysis of the VAC-SITE revealed a highly-inflamed granulomatous structure, with a necrotic center encircled by CD11c+ nested-clusters, surrounded by CD8+ lymphocytes, mostly PD1-negative and scarce FOXP3+ lymphocytes. Large numbers of Langhans multinucleated-giant-cells and macrophages were as well detected. The C-MTS contained a large tumor-regression area fulfilled with brisk lymphocyte infiltration, mainly composed of CD8+PD1+ T-cells, CD20+ B-cells, and scarce FOXP3+ cells. Despite the emergence of a loco-regional lymph-node and a small cutaneous metastasis, patient-045 remains disease-free 36 months after initiating CSF-470 vaccination. The immune response induced throughout treatment was evidenced by increasing DTH score and IFN-γ ELISPOT assay against the vaccine lysate. TCRβ repertoire analysis revealed for the first time the presence of common clonotypes between the VAC-SITE and the C-MTS; most of them persisted in blood by the end of the immunization protocol. We found that expansion of such persistent clonotypes might derive from two different although complementary mechanisms: proliferation of specific clones as well as expansion of redundant clones, which increased the number of nucleotide rearrangements per clonotype, suggesting a functional antigenic selection. In this patient, immunization with the CSF-470 vaccine plus BCG and rhGM-CSF induced a T-cell repertoire at the VAC-SITE that was able to infiltrate an emerging C-MTS, contributing to the generation of a local immune response, resulting in the expansion of a T-cell repertoire which persisted in blood by the end of the 2-year treatment.

Keywords: CUTANEOUS MELANOMA, CSF-470 VACCINE, ADYUVANT SETTING, TCRB REPERTOIRE.

## A054 / SOX2-targeted T cell therapy in multiple myeloma

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Multiple myeloma (MM) results from the uncontrolled growth of clonal antibody-producing plasma cells in the bone marrow. It is the second most common hematologic malignancy in the United States, and is nearly always fatal. Adoptive cell therapy (ACT) is emerging as a promising treatment for MM, but it requires further improvement and mechanistic understanding prior to becoming a reproducibly effective and/or curative strategy. Chimeric

antigen receptor (CAR) T cells targeting CD19, BCMA, or CD138 antigens expressed on MM cell surface have demonstrated therapeutic benefit in some patients. Unfortunately, many patients have relapsed due to the outgrowth of antigen-negative MM cells. These observations suggest that targeting a protein involved in the induction or maintenance of the malignant phenotype may be critical for the success of ACT. Recent studies suggest that the transcription factor SOX2 may regulate cell proliferation and self-renewal in MM and other cancers. Spontaneous T cell immunity to SOX2 in MM patients has been associated with slower progression after standard therapy, and with a prolonged response after treatment with anti-CD19 CAR T cells. We have therefore hypothesized that adoptively transferred T cells targeting SOX2 will have a clinical benefit in MM. To test this hypothesis, we have begun isolating high affinity SOX2-specific T cell receptors (TCRs) from healthy HLA-A*02:01 donors. To date, we have generated 40 T cell lines that recognize six distinct SOX2 epitopes in the context of HLA-A*02:01 with varying affinities. We confirmed that T cells from these lines can respond to endogenously processed and presented SOX2 peptides, suggesting that they might have anti-tumor efficacy. We will next sort the highest affinity SOX2-specific CD8+ T cells from these lines for sequencing and cloning the TCRs. Assembled TCR pairs will be tested in vitro for specific tumor cell killing, and in vivo in a humanized mouse model for safety and anti-myeloma efficacy. Collectively, these experiments should reveal if SOX2 is a safe and effective T cell target for treating MM.

Keywords: myeloma, T cell receptor, adoptive transfer.

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## A055 / Novel microbial-based immunotherapy approach enables application of adoptive cell therapy for solid tumors by enhancing both immune access to tumor microenvironment and cancer cell immunogenecity

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This work describes the development of a novel platform of microbial-based immunotherapies, called Site-Specific Immunomodulators (SSIs), that consistently recruit activated anti-cancer immune effector cells into solid tumors in specific organ sites while simultaneously overcoming immune suppression in the tumor microenvironment. SSIs are inactivated microbial-based immune modulators that are formulated from a bacterial species that is an endogenous cause of infection in the specific organ where the pathology exists, and they are easily self-administered subcutaneously by the patient. The development of SSIs has overcome many of the issues that have plagued microbe-based cancer treatments to date. Clinical application of the relationship between acute infection and cancer inhibition has been well documented historically. Dr. William Coley has been credited with bringing this insight to the attention of Western Medicine in the late 1800's when he noted spontaneous tumor remission in some patients experiencing acute microbial infections. The mechanisms driving this phenomenon have lacked sufficient characterization, and the ability to harness it safely and consistently has been elusive. As a consequence, the greater potential of microbial-based immunotherapeutic strategies have yet to be fully realized in clinical application to fight malignancy. The primary exception is the use of Bacillus Galmette-Guerin (BCG) for the treatment of high-risk non-muscle invasive bladder cancer, which is the only microbe-based therapy that is currently part of the approved standard of care. We have shown treatment with SSIs markedly increases the number of phagocytic leukocytes in circulation, shifts myeloid cell polarization to anti-cancer in function, up-regulates NKG2D ligands on tumor cells, and heightens cytotoxic lymphocyte recruitment and activity in the tumor microenvironment [1]. In this new work, we demonstrate that these collective anti-cancer functional properties of SSIs overcome the limitations of adoptive cell therapies, such as CAR T cells, in solid tumors. Using three different solid tumor models, representing lung and ovarian cancers, combination treatment with SSIs is shown to enhance the infiltration and efficacy of adoptively transferred tumor-specific transgenic T cells. In these models, the increased anti-cancer efficacy is in large part due to SSI-induced secretion of chemokines from both the tissues surrounding the tumor as well as the tumor cells themselves. These results demonstrate that SSIs can successfully overcome the major hurdles adoptive cell therapies have faced in treating solid tumors, specifically in relation to inadequate infiltration of the effector cells into the tumor and the immune suppression that is inherent in the tumor microenvironment.

In conclusion, this work demonstrates that SSIs have significant potential in transforming the treatment of solid tumors, especially when used synergistically in combination with adoptive cell therapies.

Keywords: microbial-based therapies, adoptive cell therapy, CAR T cells, solid tumors.

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## A056 / Smac mimetic (SM) induced sensitization of rhabdomyosarcoma cells towards natural killer cell mediated killing

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Rhabdomyosarcoma (RMS) is one of the most common form of pediatric soft tissue cancers. Although the overall five-year survival rate has increased over the last decades, the more aggressive metastatic alveolar RMS subtype still has a poor outcome with an overall five-year survival rate below 20%.

One underlying mechanism of cell death resistance and cancer progression in RMS is a dysregulation of inhibitor of apoptosis proteins (IAPs). Small molecule inhibitors mimicking the endogenous IAP antagonist Second mitochondria-derived activator of caspases (Smac), i.e. SM, can restore the apoptosis inducing pathways of cancer cells.

In our previous work, we could describe that the Smac mimetic BV6 influences both effector and target cells simultaneously, enhancing the susceptibility of tumor cells to NK cell-mediated killing on the one hand and increasing the cytotoxic activity of NK cells on the other hand.

To elucidate the underlying mechanisms of SM induced sensitization in RMS cells activation of signaling pathways was investigated using western blot analysis and quantitative real time PCR (qPCR). Further, flow cytometry was used to analyze the surface expression of death receptors on either SM treated or untreated RMS cells. The overall effect on cell death induction was measured by PI/Hoechst staining using a fluorescence microscope. To investigate NK cells as therapeutic strategy for treatment of RMS, co-culture experiments of RMS cells with NK cells were performed.

Notably, pretreatment of RMS cell with SM significantly increased the NK cell-mediated killing of RMS cells. Treatment of RMS or NK cells with SM led to a rapid degradation of cIAP1 and cIAP2 and in turn activated the non-canonical NF-κB signaling pathway, indicated by the accumulation of NIK and degradation of p100 to p52. Additionally, the canonical NF κB signaling pathway was activated in RMS cells, as shown by the phosphorylation of IκBα and

p65. Determination of selected target gene transcription in RMS cells revealed an upregulation of the inhibitor IκBα, NIK, p100, IL-8 and at later time points the death receptors TRAIL-R1 and TRAIL-R2. To evaluate the involvement of TRAIL-R1 and TRAIL-R2 in SM induced sensitization towards NK cell-mediated killing, surface expression of both death receptors was analyzed, and identified an increased surface presentation exclusively of TRAIL-R2. In addition, cell death analysis revealed a synergistic induction of cell death by a SM-TRAIL co-treatment.

On a transcriptional level, NK cells significantly downregulated TNFα and showed an increased IFNγ transcription upon SM treatment. Furthermore, SM seem to influence the NK cell subpopulation phenotype and might thereby modulate the NK cell activity.

The aforementioned analysis of gene transcription in RMS cells hints towards a bimodal feedback mechanism regulating both, the canonical and non-canonical NF-κB signaling pathway. On the one side, the canonical pathway is negatively regulated by the induced transcription of the inhibitor IκBα. On the other side, the induced transcription of NIK, p100 and RelB points towards a positive feedback loop of the non-canonical pathway.

The increased TRAIL-R2 expression upon SM treatment provides a mechanistic explanation for the observed SM induced sensitization of RMS cells towards NK cell mediated killing. Co-culture experiments in which a pre-blocking of TRAIL on NK cells diminished the cytotoxic effect on SM sensitized RMS cells, further support a TRAIL receptor dependent mode of killing.

Finally, based on the understanding of molecular mechanisms, the therapeutic potential of this combination can be evaluated in preclinical RMS models, thus aiming to develop a combinatory Smac mimetic – NK cell based immunotherapeutic approach.

Keywords: NK cell, Rhabdomyosarcoma, Smac mimetic, TRAIL.

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## A057 / Generation of CDK4(R24C)- and CSPG4-specific chimerized αβTCRs using a single universal bicistronic vector backbone

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We recently developed an approach for simultaneous cloning and chimerization of αβTCRs based on a single universal bicistronic vector backbone. In the present study we validate our procedure and describe the cloning of chimerized TCRs against mutated cyclin-dependent kinase 4 (CDK4(R24C)) and the melanoma-associated antigen chondroitin sulfate proteoglycan 4 (CSPG4) restricted by HLA-A*02:01 or HLA-C*07:01, respectively. The parental T cell clones, CTL14/35 against CDK4(R24C) and CTL11C/73 against CSPG4, had been derived in our laboratory from autologous mixed lymphocyte-melanoma cell cultures. The Gateway® pDONR221 entry vector backbone harbors the murine codon-optimized TCRα and β constant domains separated by a P2A element. Two head-to-head-oriented type IIS restriction enzyme recognition sites for BsmBI (TCRα) and BsaI (TCRß) precede these constant domains. The generation of chimerized αβTCRs against CDK4(R24C) and CSPG4 required (1) to amplify DNA sequences coding for the V(D)J regions of the α and ß chains of both TCRs flanked with BsmBI- or BsaI-compatible ends and (2) to perform directional seamless ligation into the vector backbone in a two-step assembly approach. With this procedure we were able to rapidly generate entry clones harboring bicistronic cTCRs in 3-4 days. Following Gateway® recombination into a retroviral pMX-IRES-puro expression vector, the cTCRs were transduced into CD8+ T cells for further in vitro testing. This resulted in uniform expression of our cTCRs in up to 96% of the transduced T-cell populations following short-term expansion under puromycin selection pressure. Functionality was verified with IFNγ-ELISpot and CD107a-degranulation assays. Target cells were melanoma cell lines expressing CDK4(R24C) or CSPG4 as well as HEK293T or COS-7 cells transiently transfected with the restricting HLA class I alleles and either co-transfected with titrated amounts of antigen-encoding cDNA or pulsed with target peptides. We demonstrated that the specificity and sensitivity of transgenic T cells was at least equivalent to the parental T-cell clones mentioned above. Collectively our results confirm the utility of our cloning procedure to rapidly generate highly functional αβTCR-transgenic T cells for further preclinical evaluation.

Keywords: T-cell receptor cloning.

## A058 / MDSC suppression of CAR T cells can be reduced by targeted signaling disruption

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CAR T cells are genetically modified with an extracellular scFv, transmembrane domain, and intracellular costimulatory and CD3zeta domains. Two treatments received the approval from the FDA for the treatment of acute lymphoblastic leukemia and diffuse large B cell lymphoma. However, CAR T cell persistence remains a problem. A reason for this may be that myeloid cells such as myeloid derived suppressor cells (MDSCs) may be contributing to the reduced persistence of CAR T cells. MDSCs originate from myeloid cells and have been implicated in the suppression of the immune system in the tumor microenvironment. To determine what effect MDSCs might have during CAR T cell production we co-cultured MDSCs during CAR transduction. We found gene transfer was lower for m19z (38.7% vs 46.8%), m1928z (24.1% vs 39.1%), and m19hBBz (35.8% vs 46.2%) CAR T cells co-cultured with MDSCs compared to those that were not. There was also a reduction in total T cell counts for m19z (58%), m1928z (88%), and m19hBBz (65%) after MDSC co-culture. This data suggests MDSCs present during CAR T cell production can alter gene transfer and total T cell counts. We also investigated the effect MDSCs can potentially have on CAR T cells when present during CAR T cell antigen stimulation. CAR T cells co-cultured with MDSCs in vitro had significant reductions in m19z, m1928z, and m19hBBz CAR T cell mediated killing against target cells. We also found significantly lower production of IFNgamma in m19z, m1928z, and m19hBBz CAR T cells cultured with MDSCs compared to cells cultured with total BM. After 24hr stimulation with MDSCs and target cells, there was lower expression of activation markers PD1 and LAG3 by CAR T cells compared to culture without MDSCs.

This suggests that MDSCs reduce CAR T cell activation, killing, and cytokine production. We also looked at how CAR proliferation after antigen stimulation is affected by MDSC co-culture and found MDSCs significantly reduced CAR proliferation in vitro. To evaluate if we could create a more resistant CAR T cell to MDSC suppression null mutations were incorporated into a CD28 CAR. We mutated the YMNM and PRRP subdomains of CD28 which signal through PI3K and ITK respectively leaving only PYAP active (mut06). When MDSCs were co-cultured with mut06 T cells during production mut06 had a smaller reduction in gene transfer (21% vs 38%) and T cell counts (80% vs 88%) compared to m1928z. Mut06 also had a significantly higher expression of PD1 and TIM3 compared to m1928z after production with MDSCs. In vitro when mut06 was co-cultured with MDSCs it had the same killing ability as m1928z without MDSCs and was significantly better at killing compared to m1928z co-cultured with MDSCs. To examine the effect of MDSCs on CAR T cells in vivo we injected C57BL6 mice with CAR T cells followed by MDSCs a week later. In these ongoing experiments we found mut06 had significantly higher numbers of CAR T cells in the blood compared to m1928z. Overall our data shows that MDSCs can suppress CAR T cell function when present during production as well as CAR stimulation. It also suggests that by optimizing CD28 CAR signaling using mut06 that we were able to generate a CAR T cell that is more resistant to MDSCs. Furthermore, we may be able to recapitulate the effect of mut06 with targeted inhibitors.

Keywords: Chimeric Antigen Receptor, Myeloid Derived Suppressor Cells.

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## A059 / Cancer cell defects in intrinsic apoptosis attenuate killing by CAR T cells

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Chimeric antigen receptor (CAR) T cell therapy is now an FDA approved treatment for several hematologic malignancies, yet not all patients respond to this treatment (1). While some resistance mechanisms have been identified, the possibility that cell death pathways in target cancer cells could impact response to CAR T therapy remains underexplored. To assess whether cell death pathways in target cancer cells could impact response to CAR T therapy, we utilized a HeLa in vitro model system. HeLa cells with intact (HeLa-19) and deficient Bak/Bax (HeLa-DKO-19) expressing CD19 were co-cultured with CD19 CAR T cells. Viability of target cells was then quantified using a) Annexin V / Hoechst staining and b) an impedance-based readout of cell death. We observed that Bak/Bax deficiency, which blocks the intrinsic pathway of apoptosis, conferred resistance to CAR T killing. However, this resistance could be overcome at high E:T ratios. To confirm that the intrinsic pathway of apoptosis contributes to CAR T killing, we forced the expression of Bcl-2 and Bcl-XL in HeLa-19 cells, and observed that both of these anti-apoptotic proteins conferred protection from CAR-T killing in a similar manner to Bak/Bax knockout. Additionally, we wanted to assess whether caspases may be required for CAR T killing, given the role of intrinsic apoptosis in our model system. The caspase inhibitor Z-VAD-FMK protected both HeLa-19 and HeLa-DKO-19 cells from CD19 CAR T effector cells in our in vitro model system. Lastly, we wanted to ascertain the precise mechanism by which CAR T cells eliminate target cancer cells. CAR T cells were co-cultured with HeLa-19 and HeLa-DKO-19 targets in the presence of blocking antibodies against the death ligands FasL and TRAIL; or 3, 4-dichloroisocoumarin (DCI), a granzyme inhibitor. We observed that granzyme inhibition, but not Fas ligand and TRAIL blockade, provided target cells with protection from CAR T cells. Additionally, a soluble factor contributed to CAR T killing of HeLa-19 target cells, as demonstrated by conditioned media experiments in which target cells were exposed to filtered supernatant from CAR T co-culture experiments. We tested whether death ligands (FasL, TRAIL, Tnf-α) were responsible for conditioned media killing using blocking antibodies as described above. Tnf-α blockade, but not FasL and TRAIL blockade, protected HeLa-19 target cells from conditioned media.

In conclusion, intrinsic apoptosis allows for efficient elimination of target cancer cells by CD19 CAR T cells. Similarly, caspase inhibition with Z-VAD-FMK increased the number of CAR T cells required for efficient killing. This killing seems to be mediated largely by granzymes, as well as by Tnf-α. Our work suggests that tumor cells that are resistant to intrinsic or downstream apoptosis may resist CAR T therapy. Future experiments will explore whether the intrinsic apoptotic pathway also modifies response to CAR T cells in a mouse model; and will identify additional soluble factors that contribute to CAR T killing of target cells.

Keywords: CAR T cell, CTL, apoptosis, cancer.

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## A060 / The hypoxia-inducible metabolite S-2HG increases the memory potential of CAR-T cells

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Cancer immunotherapy is advancing rapidly, and the use of gene-modified T cells that express tumour-specific chimeric antigen receptors (CARs) is an aspect of these therapies that has shown particular promise. A current challenge of CAR-T cell therapy is that the ex vivo generated CAR-T cells become exhausted during expansion in culture, and do not persist when transferred back to patients. Also, CAR-T cell exhaustion and short-term immuno-surveillance limit their clinical potential against solid tumours. Less differentiated T cells, such as central memory T cells (TCM), display better expansion, anti-tumour activity and persistence, which are necessary features both for therapeutic success and for prevention of disease relapse. We have recently reported that mouse T cells express memory-like markers when treated with a Hypoxia Inducible Factor (HIF) - inducible immunometabolite, 2-hydroxyglutarate (2HG) (Tyrakis et al., Nature 2016). We hypothesised that this feature of 2HG could be evolutionary conserved and ex vivo 2HG treatment of human CAR-T cells could increase both CAR-T cell persistence and anti-tumour activity. We conducted in vitro experiments where we treated human T cells with the two 2HG enantiomers (S-2HG and R-2HG). We observed that the two enantiomers result in specific changes in T cell homeostasis and fate. T cells were more sensitive to R-2HG than to S-2HG both in cell numbers and in viability. Additionally, although both enantiomers increased the percentage of TCM cells, only S-2HG showed a significant increase in the TCM cell number. In contrast to S-2HG, R-2HG vastly decreased the expression of the CD62L homing marker and increased the PD-1 exhaustion marker. The two enantiomers also showed differences in specific epigenetic marks (histone methylation / acetylation). Our data provide evidence suggesting that treating T cells specifically with S-2HG (instead of R-2HG, which is commonly studied) can improve T cell function upon immunotherapy. We generated CAR-T cells from healthy donors and treated them with S-2HG. We observed an accumulation of central memory CAR-T cells in response to S-2HG treatment, which didn't affect the cytotoxic potential and the cytokine release of the CAR-T cells when co-cultured with target tumour cells. Our data provide evidence suggesting that treating T cells with S-2HG could alter T cell fate by coordinating the level of activation/inhibition of various targets and could improve as such T cell function upon immunotherapy.

Keywords: CAR-T cells, central memory T cells, Hypoxia Inducible metabolites.

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## A061 / A novel T cell receptor targeting a shared FLT3 mutation in acute myeloid leukemia

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Acute myeloid leukemia (AML) is the most frequent leukemia in adults. As allogeneic hematopoietic stem cell transplantation still remains the major curative option upon relapse from chemotherapy, there is a large unmet medical need for this patient group, calling for improved therapeutic strategies. Adoptive therapy with T cells genetically modified to express immune receptors targeting antigens encoded by shared mutations could be an attractive option. The FMS-like tyrosine kinase 3 (FLT3) is mutated in approximately 30% of AML patients. These mutations can be divided into two categories; (1) internal tandem duplications (FLT3/ITD mutations), and (2) point mutations resulting in single amino acid substitutions in the activation loop of the tyrosine kinase domain (FLT3/TKD mutations, 5-7% of AML). Although the ITD mutations are more frequent than the TKD point mutations, they occur in different areas resulting in different amino acid sequences in individual patients. Mutations in the D835 position are screened for in routine diagnostics, as they have prognostic importance and lead to constitutive activation of FLT3. We therefore focused on the identification of T cell receptors (TCRs) targeting the shared FLT3 point mutation D835Y, restricted by the frequently expressed HLA-A*02:01 allele. To this end, we followed our previously described strategy. Monocyte-derived dendritic cells from HLA-A*02:01(HLA-A2) positive healthy donors were electroporated with mRNA encoding the mutated sequence and co-cultured with autologous CD8+ T cells. T cells reactive to the FLT3/TKD D835Y mutation were identified by dual color tetramer staining, and were sorted for subsequent cloning. A TCR was identified from clones reactive to HLA-A2pos target cells in presence, but not in absence, of loading with the relevant peptide. Peripheral blood T cells redirected with the FLT3 TKD D835Y/HLA-A2-reactive TCR (FLT3 TCR) efficiently and specifically recognized target cells pulsed with mutated but not wild type peptide at concentrations as low as 10-100pM, indicating high functional avidity. No reactivity was observed against a panel of 15 HLA-A*02:01 positive cell lines of different tissue origins and expressing a variety of HLA types, unless the cells were pulsed with the mutant peptide or electroporated with relevant mRNA, indicating high peptide- and HLA-specificity. TCR-transduced T cells also reacted vigorously to patient leukemia cells harboring the relevant mutation, whereas no reactivity was observed in response to HLA-A2pos patient leukemia cells carrying the D835E mutation or the FLT3/ITD mutation, or to HLA-A2neg cells expressing the targeted mutation. Importantly, in vitro killing assays showed that 91% 3 3.6% (mean 3 SEM, n=3) of the tumor cells specifically disappeared in samples from three different patients during the span of 72 hours upon treatment with FLT3 TCR-transduced but not mock-transduced T cells, whereas no difference was observed in the numbers of normal T and B cells in the same patient samples when comparing treatment with FLT3-TCR and mock-TCR-transduced T cells. At end of co-culture, we next investigated if the few remaining immature patient myeloid cells falling in the same flow cytometric gate as the leukemic blast cells harbored the targeted mutation or not. PCR of sorted single cells demonstrated that indeed all but one among a total of 77 sorted cells from three patients expressed either the wild type counterpart of the target or the alternative D835E mutation.

In conclusion, we have identified a TCR that is highly specific for a peptide encoded by the recurrent AML-associated FLT3/TKD D835Y mutation presented on HLA-A2, that efficiently eliminates primary leukemia cells harboring this mutation when transduced into primary T cells. The results call for further preclinical testing in preparation for potential clinical application.

Keywords: Immunology, Immunotherapy, Cancer, Leukemia.

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## A062 / Spatiotemporal coordination of T-bet expression during type 1 responses

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CD4 T cells are central orchestrators of the protective immune responses against different types of pathogens and non-microbial challenges. The principal CD4 subsets -TH1, TH2, TH17, and TFH - are distinguished by specialized gene expression features established under the control of differentially expressed lineage-defining transcription factors. The latter include expression of distinct homing and chemokine receptors, therefore, each subset likely traffics to discrete regions within the secondary lymphoid organs. The production of signature cytokines by CD4 lineages affects the activation and recruitment of other immune cell types. Consequently, by localizing to distinct sites within the secondary lymphoid organs these cells likely create microenvironments, a feature that may be critical for efficient immunity against corresponding classes of immune challenges. The principal transcription factor guiding TH1 differentiation T-bet is unique among the lineage defining factors of TH cell differentiation is also expressed in a wide range of immune cells. Using airway influenza infection as a model, we observe coordinated expression of T-bet in key lymphocyte lineages: CD8 T cells, TH1 cells, TFH cells, Treg cells and germinal center B cells. We find that these populations localize in close proximity in distinct regions within the lung draining lymph node. Ours data suggests that T-bet expression in different lymphocyte lineages coordinates formation of a spatiotemporal circuit to enable effective type 1 responses.

Keywords: T-bet.

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## A063 / Immunomodulated autologous cancer immunotherapy for treatment of prostate cancer

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Despite improvements in surgical, radiation, and other therapies for localized prostate cancer, up to 30% of patients will unfortunately experience recurrent prostate cancer after prostatectomy. FK-PC101 is a novel immunotherapy, when added to prostatectomy and other standard treatments for localized prostate cancer, may reduce the incidence of recurrent disease. FK-PC101 is

a product consisting of autologous human prostate cancer cells which have been modified to express MHC II on their surface. Upon injection of the cells into the subject from whom they were originally isolated, the modified cancer cells will not only present a cancer neoantigen in the context of MHC I to naïve (unprimed) CD8+ cytotoxic T cells; but also present antigens to CD4+ helper T cells in the context of MHC II. This study is intended to evaluate the incidence of PSA recurrence in the vaccine group and compared to a concurrent control group. Methodology: A two-step process was carried out for screening and enrollment. A total of 106 subjects were consented and underwent radical prostatectomy at a single institution in Brazil. A total of 62 subjects with high risk features based on clinical-pathologic findings from the prostatectomy were enrolled. Of the 62 subjects enrolled, 23 subjects received immunotherapy. The remaining 39 subjects were not immunized and were included as control group. FK-PC101 administration was started approximately 3-4 months following prostatectomy, intradermal injections were performed days 0, 7, 14, 21, 51, 81 and 171. Baseline demographic features were similar between the two groups. Prostate cancer risk features, including high Gleason grade and higher pathologic cancer stage, were more common in the vaccine group. The reported use of hormonal therapies, radiation therapy, and other therapies post-RP were similar between the two groups. Median follow-up times were also similar in both groups. Median duration of follow-up was 11.1 years (range 2.8, 15.4 years) in the vaccine group and 9.6 years (range 1.0, 16.5 years) in the control group. Efficacy: Although the vaccine group had higher risk prostate cancer features, subjects who received the FK-P-101 immunotherapy following prostatectomy demonstrated a delay in time to PSA recurrence. The median time to PSA recurrence in the vaccine group was 104.6 months vs. 77.4 months in the control group, which represents an improvement of over 27 months (p=0.1229, log-rank test, HR=0.56). In addition, there was a reduction in the incidence of PSA relapse, for example at 3- 5 years post-RP. At 4 years post-RP, 11.8% of the vaccine group had experienced PSA recurrence, vs. 36.8% in the control group (p=0.0453, z-test). Furthermore, 1/23 (4.3%) vaccine subjects died from recurrent, progressive prostate cancer, compared to 5/39 (12.8%) control group subjects. Safety: The most common AE's reported in the vaccine group included local injection site reactions which were mild to moderate in severity and generally resolved within a few days. There were no Grade 3 or higher injection site AE's or reactions. There were no generalized or systemic reactions to the injections, and there did not appear to be any latent reactions. None of the vaccine subjects had vaccine therapy discontinued due to AE's.

Conclusion: Based on the evaluation of the PSA recurrence data, there appears to be a decrease in the rate of PSA recurrence in the immunized group. In particular, there was a significant decrease in the incidence of PSA recurrence at 4 years in the vaccine group (11.8% vs. 36.8% p=0.0453, z-test). These findings, coupled with favorable trends in survival and the reported safety profile, support further evaluation of this immunotherapy approach. Following a pre-IND meeting with the FDA (May/2019), we are planning an adaptive Phase 2b/3 randomized trial of FK-PC101 in patients who have undergone prostatectomy for unfavorable intermediate or higher risk prostate cancer.

Keywords: Immunotherapy, Prostate Cancer, Tumor Presenting Cells, Phase 2.

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## Immunotherapies, non-cell-based

### A064 / Identification of tolerant tumor neoepitopes drives predictions of response to immune checkpoint blockade therapy

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Effective T cell reactivity against tumor neoepitopes is proposed as a key mechanism in immune checkpoint blockade (ICB) therapy. Therefore, defining rules for the immunogenicity of neoepitopes has been the subject of multiple recent studies and tumor mutational burden has been demonstrated to function as a potential biomarker for predicting response to ICB therapy. However, only a small fraction of predicted neoepitopes can elicit immune responses in vitro and in vivo. Therefore, the identification of the non-immunogenic (tolerant) subgroup of neoepitopes could have an important impact on target identification for neoepitope-based therapies, such a patient specific vaccines, as well as on predicting responses to ICB therapy. In order to identify those tolerant neoepitopes we hypothesized that a patient's T cell reactivity against their tumor's neoepitopes will be limited by pre-existing T cell tolerance to non-mutated, normally presented human leukocyte antigen (HLA) ligands. To test this hypothesis, we developed a model to predict tolerance against neoepitopes based on their physicochemical similarity to over 169, 000 non-mutated 9-mer HLA class I ligands identified by mass spectrometry (MS). We trained our model on a subset of 92 published neoepitopes and validated the approach with a test set of 345 mutated HLA ligands. Our model prospectively identified non-immunogenic neoepitopes with high positive predictive value (97%) independently of their predicted binding affinity. The algorithm also incorporates a novel mechanism, which we term "cross-allelic tolerance". This mechanism is based on the assumption that high similarity between a neoepitope and a non-mutated self-peptide at their T cell receptor recognition areas can be sufficient to confer tolerance to the neoepitope independent of its presenting HLA allele, but dependent on the HLA allele repertoire of the patient. Furthermore, utilizing these Methods to incorporate the non-immunogenicity of a large fraction of neoepitopes, we define the "RESPONDER" score, which predicts patients' responses to ICB therapy with unprecedented precision in melanoma and non-small cell lung cancer. In a cohort of 126 melanoma patients, 69 patients with a low RESPONDER score had a median overall survival (mOS) of 9.7 months, whereas the subgroup of 57 patients with a high RESPONDER score achieved mOS of 48.6 months (TMB alone as a biomarker in this cohort defines subgroups with mOS of 10.8 vs. 23.4 months).

Altogether, our model prospectively predicts tolerance to neoepitopes, as well as response to ICB therapy in multiple cancer entities with very high precision utilizing a novel explanation for tolerance to certain neoepitopes, termed cross-allelic tolerance. Therefore, this approach should facilitate the design of neoepitope-based therapies and spare potentially unresponsive patients from toxicities and costs of ICB therapy.

Keywords: HLA ligand, Neoepitope, Immune Checkpoint Blockade, Response Prediction.

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## A065 / Inhibition of IGSF11 mediates efficient tumor cell killing in vitro and in vivo

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Immune-checkpoint blockade by drugs targeting CTLA-4, PD-1 or PD-L1 pathways have emerged as the standard of care in many tumor indications, however it still doesn't meet the needs of a majority of cancer patients. To understand and discover alternative druggable vulnerabilities in immunosuppressive tumors, we used our iOTarg™ genetic screening platform to identify targets in a lung cancer model that fails to respond to PD-L1 inhibition. Immunoglobulin superfamily member 11 (IGSF11) was identified as a hit from our iOTarg™ screen, whereby its knockdown led to a significant increase in tumor cell killing by lung cancer patient-derived tumor-infiltrating lymphocytes (TILs). IGSF11 is a type I transmembrane cell adhesion molecule that was recently described as a putative ligand of V-domain Ig suppressor of T cell activation (VISTA). IGSF11 expression in healthy tissues is largely restricted to immune-privileged organs such as brain, ovaries and testis, whereas it is frequently upregulated in various types of cancer. Validation studies with different tumor and T cell pairs demonstrated that siRNA-mediated downregulation of IGSF11 on tumor cell surface led to improved T cell activity and tumor cell killing. We raised a diverse panel of fully human antibodies against human IGSF11 using phage display technology. Antibodies were characterized by binding to recombinant protein as well as to IGSF11-expressing tumor cell lines and those with human/mouse cross-reactivity were further tested for immune modulatory potential in in vitro killing assays. For that purpose, recombinant IGSF11 overexpressing MDA-MB-231 human breast cancer cells or endogenously expressing human melanoma cells were cocultured with T cells isolated from healthy donor PBMCs in the presence of anti-IGSF11 antibodies. Tumor cell viability was determined after three days using the CellTiter-Glo viability assay. A defined subset of epitope-specific antibodies were identified that mediated strong, dose-dependent killing of tumor cells. The tumor lysis was target- and T cell-dependent, as sequestration of antibodies by exogenous addition of recombinant antigen or absence of T cells abolished this effect. To further corroborate our in vitro findings, we engineered MC38 mouse colon adenocarcinoma cell line for overexpression (OE) or CRISPR/Cas9-mediated knock-out (KO) of mouse Igsf11. C57BL/6 mice with subcutaneously implanted KO tumors showed significant reduction in tumor outgrowth compared to wild type (WT) tumor and OE tumor-bearing mice. In line with reduced tumor burden, KO tumors exhibited a trend towards higher infiltration of T cells (including cytotoxic T cells) and a stronger reduction of granulocytic MDSCs as compared to the OE tumors. In summary, we demonstrate that tumor-expressed IGSF11 is a T cell-inhibitory molecule whose immunosuppressive activity can be abrogated by specific monoclonal antibodies to achieve efficient tumor cell killing. Using a syngeneic mouse model, we further validate the relevance of IGSF11 in shaping antitumoral immune responses in vivo. IGSF11 is a promising drug target for cancer treatment, especially for patients that have relapsed or are non-responsive to classical immune-checkpoint inhibitors.

Keywords: Immune-checkpoint, High-throughput screen, IGSF11.

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## A066 / Electrochemotherapy in combination with peritumoral gene electrotransfer of interleukin-12 as an adjuvant immunotherapy varies according to immune status of treated tumors

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Although the therapeutic effectiveness of electrochemotherapy (ECT) in the clinics is up to 80% of local tumor control, systemic antitumor effect (i.e. abscopal effect) has not been observed. With the aim to achieve systemic effect, we proposed a combination therapy consisting of ECT with intratumoral application of cisplatin, oxaliplatin or bleomycin with peritumoral gene electrotransfer (GET) of plasmid encoding interleukin-12 (IL-12). In the combination, IL-12 boosts the in situ vaccination effect of ECT and together with immunogenic cell death and danger-associated molecular patterns release recruits effector immune cells. Therefore, we hypothesized that IL-12 potentiates the effect of ECT on a local and systemic level and may vary regarding immunological profile of the treated tumors. For this purpose, the combined therapy was tested in three immunologically different murine tumor models (malignant melanoma (B16F10), colon carcinoma (CT26) and mammary carcinoma (4T1)). The tumor growth was followed on primary treated tumors (B16F10, CT26, 4T1) and on distant untreated tumors using dual-flank model mimicking systemic disease. The therapy was performed when tumors reached 35-40 mm3 in volume. Additionally, tumor and blood samples were collected for immunohistochemical and cytometric analysis of the tumor microenvironment and for the detection of IL-12. First, we investigated the effect of ECT in B16F10 melanoma using equally effective but low doses of cisplatin, oxaliplatin or bleomycin in combination with peritumoral GET of IL-12. IL-12 potentiated the antitumor effect of ECT with all three drugs. However, we observed the most pronounced potentiation with ECT using cisplatin, resulting in 38% of complete responses as well as an abscopal effect. The antitumor effectiveness of this treatment combination could be ascribed to the induction of the local and systemic immune response. Namely, infiltration of granzyme B positive immune cells was observed in both, primary and distant tumors. Furthermore, the combination therapy was also tested in two other immunologically different tumor models, CT26 and 4T1. The results indicate that peritumoral GET of IL-12 inversely correlates with tumor response to ECT. We observed better responsiveness to ECT in immunologically "hot" tumors, where the addition of GET led to the lowest potentiation. To conclude, peritumoral GET of IL-12 significantly potentiates ECT in treated melanoma tumors and has some effect on distant untreated tumors, predominantly when cisplatin was used for ECT. Effectiveness of the tested treatment combinations depends on the immunological status of the tumor. ECT was more effective in immunologically "hot" tumors but the contribution of peritumoral GET was not as pronounced as in "cold" tumors.

Keywords: electrochemotherapy, gene electrotransfer, interleukin-12, tumor immunological status.

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## A067 / Tracking oncolytic virus-specific CD8+ T cells with epitope-based, HLA-agnostic peptides in a renal cell carcinoma clinical trial

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Oncolytic viruses are engineered to preferentially replicate within and kill tumor cells, inducing anti-tumor immune responses. While monotherapy treatment with oncolytic viruses has shown limited clinical response in some cancers, their full immune-stimulating mechanisms have not been elucidated. A critical component to delineate the anti-tumor activity of oncolytic viruses is to monitor peripheral immune responses to both the virus and the tumor. In the study described here, we developed assays to monitor functional CD8+ T cell responses in the blood of patients treated with the Vaccinia-based oncolytic virus by SillaJen, Pexa-Vec. Patients with metastatic renal cell carcinoma (RCC) refractory to standard of care treatments were treated intravenously with 109 PFU Pexa-Vec once a week for five weeks, after which treatment switched to once every three weeks for the duration of study. PBMC were collected and cryopreserved prior to treatment, at six and twelve weeks post-Pexa-Vec initiation, and upon completion of study. Clinical response was determined for all patients according to mRECIST1.0 criteria at weeks six, twelve, and upon study completion: Stable Disease (<20% increase and <20% decrease in lesion size), Progressive Disease (>20% increase), or Response (>20% decrease). To efficiently use small volumes of collected blood, we designed custom peptide pools from known immunogenic Vaccinia epitopes in an HLA-agnostic format to profile peripheral CD8+ T cell responses. All curated MHC class I-restricted epitopes to Vaccinia virus were retrieved from the Immune Epitope Database and duplicates were removed. The remaining epitopes (n=71) were synthesized and pooled based on known HLA restriction. Individual pools were comprised of no more than 20 peptides. PBMCs were stimulated for 48 hours, and responses to peptide pools were measured by dual IFNgamma/granzyme B ELISPOT. Additionally, patient HLA haplotypes were determined by sequencing, and all samples were evaluated by flow cytometry to assess T cell phenotypes. We found that the epitope-based, HLA-agnostic peptide pools were successful in tracking CD8+ T cell responses in RCC patients treated with the oncolytic virus Pexa-Vec. Interestingly, eight of eleven patients with Stable Disease at week six had increased responses to the peptide pools relative to baseline, whereas three out of four Progressive Disease patients displayed decreased responses ($p < 0.05$, Fisher t test). Additionally, an increase in PBMC response at week six to the A*02 supertype pool directly correlated to overall survival of the patients, whereas no correlation was detected with the other peptide pools.

In conclusion, profiling of the T cell response can be achieved with minimal sample requirements in an HLA-agnostic manner using custom peptide pools based on known Vaccinia epitopes. By reducing and improving the number of candidate epitopes (10s rather than 100-1000s), the assay can track systemic patient responses while using less of the valuable clinical samples.

Keywords: Oncolytic Virus, T cell response.

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## A068 / Systematic assessment of T cell immune checkpoint (ICP) pathways

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Background: More than twenty distinct ICPs are being evaluated as T cell targets for cancer immunotherapy. Understanding the general redundancy or specificity of ICP pathways in T cell is key for next generation immunotherapies or combinatorial trials. A detailed systematic comParison and description of their biological effector functions in T cell is lacking in the field.

Goal: Here, we aimed at providing a deep profiling and characterization of distinct T cell ICPs through systematic comParison across multi-parametric functional assays.

Methods: We selected different co-stimulatory or inhibitory ICP of interest based on the literature. Whenever possible, benchmark antibodies were identified, produced and validated for their described in vitro mechanism of action. Head to head comParison of ICP treatments were performed in vitro using human primary T cells alone or co-cultured with Dendritic Cells. Proliferation and cytokine secretion were used as readouts, using multiple donors.

Results: First, we showed that PD1/PDL1 blockade induced higher T cell cytokine secretion in both CD4 and CD8 MLR. Then, combining anti-CTLA-4 to anti-PDL1 treatments we showed improved T cell activation compared to anti PDL1 alone, which fully validated our model. Then, we found that all co-stimulatory ICP tested were able to induce high and significant CD4 T cell proliferation in combination with anti CD3 alone, in DC free experiments. In addition, specific cytokine profiles were found in response to each costim-ICP stimulation, highlighting the limited redundancy of these pathways. Specificity of cytokine patterns was also retrieved when combining costim-ICP to anti-PDL1 treatment, revealing potential new synergistic effects.

Conclusion: Our systematic analysis of ICP in T cells provides key insights about the specificity or redundancy of these pathways. Our data constitutes an important resource for optimizing drug development or combinatorial treatments, which is a key challenge of anticancer immunotherapy.

Keywords: T cell, Immune checkpoint.

## A069 / Systemic Anti-PD-1-activated doxorubicin prodrug synergizes antitumor effect mediated by accumulation of tumor infiltrating lymphocytes

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Background: We developed doxorubicin prodrug (MPD-1) activated in the presence of caspase-3. Increased antigenicity and tumor infiltrating lymphocytes (TILs) are prerequisites for maximizing the efficacy of anti-PD-1. Thus, we hypothesized that anti-PD-1 therapy induces apoptosis in the tumor and produces caspase-3, activating MPD-1. Activated MPD-1 also has cytotoxic effect and aggregates TILs in the tumor. This process would synergize the anti-tumor effect. We evaluated this positive feedback in murine squamous cell carcinoma model.

Methods: Murine SCC VII (squamous cell carcinoma) cells were inoculated to female C3H/J. We divided 6 treatment groups; control, radiation therapy, anti-PD-1, radiation, anti-PD-1 with MPD-1, RT with MPD-1, and triple therapy (anti-PD-1 + RT + MPD-1) (n = 5/group). After the tumor grew up to 200 mm3, each treatment was initiated. Tumor size and mice weight were measured. Distribution of CD45+, CD8+, CD4+, and FoxP3+ cells was analyzed. results Either RT or free doxorubicin showed the limited anti-tumor effect with higher weight loss than MPD-1+RT group. Anti-PD-1 treatment induced caspase-3, an activator of MPD-1 in the tumor microenvironment. Combination therapy using RT with MPD-1 or anti-PD-1 with MPD-1 showed better antitumor effect than monotherapy. CD45+ TILs and CD8+CD45+ TILs were increased in anti-PD-1 + MPD-1 group than RT + MPD-1 group. When CD8 was blocked by anti-CD8 antibody, tumor growth was recovered only in anti-PD-1 + MPD-1 group.

Conclusion: Systemic anti-PD-1 initiates apoptosis and produces caspase-3 activating MPD-1. It also changes the population of TILs possibly augmenting immunogenic cell death. CD8+ TILs may have a pivotal role in combination treatment.

Keywords: Anti-PD-1, Doxorubicin, SCC VII, Combination.

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## A070 / Preclinical characterization of Sym023: A human anti-TIM3 antibody with a novel mechanism of action

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Immunotherapy has become a major focus of research in oncology and blockade of immune checkpoints such as cytotoxic T-Lymphocyte Associated Protein 4 (CTLA4) and programmed cell death protein 1 (PD1) has been some of the most successful immunotherapies. The next wave of immunomodulatory targets that are being explored for cancer therapy include T cell immunoglobulin and mucin domain protein 3 (TIM3). TIM3 is constitutively expressed on cells of myeloid origin whereas the TIM3 expression is induced on T-cells upon activation. The exact function of TIM3 on the different immune cells is not clear and may be context dependent suggesting that TIM3 is not a classical immune check-point. Sym023 is a human anti-TIM3 antibody, which binds human TIM3 and cross-reacts with cynomolgus monkey TIM3. Sym023 blocks binding of phosphatidyl serine but not galectin 9 and stimulates T-cell proliferation in mixed lymphocyte reactions and tumor growth inhibition in vivo. Here, we present data demonstrating that ligation of TIM3 by Sym023 increase cytokine production and T cell proliferation in vitro through a novel mechanism of action.

Keywords: Immunotherapy, Antibody, TIM3, Dendritic cells.

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## A071 / Only STING agonists of exceptional potency can fully reprogram suppressive tumor myeloid stroma and mediate regression of established pancreatic cancer

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Immunosuppressive myeloid populations including tumor associated macrophages (TAM) and myeloid derived suppressor cells (MDSC) are abundant within pancreatic adenocarcinoma (PDAC) tumors and play critical roles in constraining cytotoxic T cell function in the tumor microenvironment. We hypothesized that intratumoral engagement of innate pathogen recognition receptors such as Stimulator of Interferon Genes (STING) could induce pro-inflammatory polarization of the myeloid stroma and liberate the antitumor T cell response to regress refractory PDAC tumors in the presence of checkpoint blockade.

We developed and characterized a novel cyclic dinucleotide (CDN) STING agonist IACS-8803, and found that 8803 activates downstream STING signaling in both human (THP-1) and murine (J774) reporter cells with over 10-fold greater potency than ML-RR-S2-CDA, the most clinically advanced CDN. Intratumoral delivery of 8803 into subcutaneous B16 melanoma and PDAC tumors additionally revealed a greater capacity to induce tumor regression relative to ML-RR-S2-CDA suggesting that therapeutic benefit of STING agonist therapy may be proportional to potency.

In order to evaluate the specific effects of STING agonists of ascending potency (c-di-GMP, cGAMP, ML-RR-S2-CDA, IACS-8803) on the phenotype and function of suppressive myeloid populations, we generated in vitro polarized human M2 macrophages and murine bone marrow-derived MDSC. We found across cytokine multiplex, 30-paramater flow cytometry, reverse-phase protein array, and gene expression analysis a clear signature of suppressive to pro-inflammatory myeloid conversion proportional to the strength of the STING agonist. While weaker agonists could reduce markers of suppressive function, they failed to generate a signature of active co-stimulatory potential. In contrast, M2 macrophages and MDSC exposed to the strongest STING agonists shed suppressive function and adopted multiple characteristics of immune-supportive function. Additionally, the capacity to functionally reverse MDSC suppression of T cell responses also appeared to be proportional to STING agonist potency.

We next investigated the capacity for intratumoral delivery of IACS-8803 to sensitize murine pancreatic cancer to checkpoint blockade and to mobilize systemic immunity against disseminated lesions. We utilized mT4-2D, a novel pancreatic cancer cell line from Kras+/LSL-G12DTp53+/LSL-R172HPdx1-Cre tumor organoids. We isolated a single cell clone of mT4-2D with reduced in vivo growth kinetics (t mT4-LS), as well as a clone which maintains the aggressiveness of the parental line (mT4-LA). Mice bearing 10-day established orthotopic and subcutaneous mT4-LS tumors received standard regimens of αCTLA-4, αPD-1, or cthe combination in the presence or absence of 8803 CDN injected into the orthotopic pancreatic tumor. We find single agent treatment with 8803, αCTLA-4, αPD-1, or αCTLA-4/αPD-1 can cure 40-60% of mice of both orthotopic and subcutaneous tumors in this system; however, combination of 8803 and checkpoint blockade completely eradicates both injected and distal mT4-LS tumors in all mice. We replicated these studies using the refractory metastatic mT4-LA model, and found that combination therapy with intra-pancreatic 8803 and systemic αCTLA-4/αPD-1 significantly extends survival compared to 8803 or αCTLA-4/αPD-1 alone ($p=0.001$, $p=0.0086$). Analysis of treated mT4-LA tumors by flow cytometry reveals that combination therapy enhances the cytotoxic potential of CD8 T cells at both injected and uninjected lesions, and promotes dendritic cell proliferation within the pancreatic milieu. These studies not only provide a pre-clinical rationale for pursuing the use of potent STING agonists as a localized approach to sensitize refractory PDAC tumors to checkpoint blockade, but also provide critical new insights into the molecular pathways engaged by innate activation to convert suppressive myeloid stroma to a pro-inflammatory phenotype.

Keywords: STING, MDSC, Pancreatic Cancer, Macrophages.

## A072 / Characterization of the first chicken derived anti-PD-1 clinical candidate antibody with a unique epitope and promising anti-cancer activity

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Inhibition of immunologic checkpoints like Programmed Cell Death 1 (PD-1) has shown clinical efficacy in a broad range of cancers by improving or restoring T-cell activity. Anti-PD-1 antibodies show great promise in treating cancer malignances when administered alone or in combination with other immune activating approaches. However, high protein sequence identity between human and mammalian species used for antibody generation often disfavor generation of antibodies against functionally conserved epitopes, or prevents isolating antibodies cross reacting with ortholog species used for evaluating potential toxicity. Chickens are phylogenetically distant from mammals and are better at generating antibodies against epitopes that are conserved in mammals. Because chickens generate antibodies from a very restricted set of V-gene germline genes that are diversified by “gene conversion”, we envisioned that high throughput humanization of antibody frameworks was achievable by “mass CDR grafting” after recovering antibodies by immunization and B-cell cloning. Wild type chickens were immunized with PD-1 antigen, and a repertoire of 120 antibodies was generated with Symplex™ technology, by combining single B-cell FACS sorting and high throughput RT-PCR cloning of cognate VH and VL chains. The isolated PD-1 repertoire was cloned with an inert Fc backbone and humanized by a combination of in silico CDR grafting and gene synthesis. Humanized antibodies were expressed and screened for retained binding affinity and functionality in T-cell based assays. We successfully generated a humanized PD-1 antibody repertoire and found that most antibodies retained affinity and functionality similar to that of parental chicken antibodies. Furthermore, the antibody repertoire displayed broad binding epitope coverage on PD-1, often with strong pM affinity, and showed biophysical properties

acceptable for drug development. Our lead antibody Sym021 blocked PD-L1 and PD-L2 ligand binding and downstream PD-1 signaling, resulting in elevated T-cell cytokine production in vitro. Moreover, Sym021 bound human PD-1 with much stronger affinity of 30 pM compared to clinical PD-1 mAbs nivolumab and pembrolizumab, while also cross reacting to cynomolgous and mouse PD-1. This enabled direct testing of Sym021 in syngenic mouse in vivo models and evaluation of preclinical toxicology in cynomolgus monkeys. Detailed epitope mapping showed that the epitope recognized by Sym021 was unique compared to clinical antibodies pembrolizumab and nivolumab. Sym021 is being evaluated in the clinic.

Keywords: Anti-PD-1, Chicken Derived.

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## A073 / Sequential and timely combination of cancer nanovaccine with an immune checkpoint blockade effectively inhibits tumor re-growth

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Although cancer nanovaccines have the advantage of increasing tumor-specific T cell pools, the real translation into clinic has been limited. Immune evasion in tumor microenvironment is one of the main causes to yield poor results in the vaccine clinical trials. In particular, adaptive resistance of the tumor induced by vaccines causes T cells dysfunction by increasing expression of immune checkpoint programmed death ligand 1 (PD-L1), which diminishes the ability of vaccines for tumor eradication. Numerous attempts have been made to combine vaccines and immune checkpoint blockade (ICB) therapy to enhance their antitumor efficacy. However, the method to optimally combine these therapeutics by considering treatment sequence and timing remains a major challenge. Here, we developed a model tumor antigen-displayed, CpG adjuvant-embeded small lipid nanoparticle (OVAPEP-SLNP@CpG)-based nanovaccine platform and also a new treatment strategy for the combination immunotherapy. The nanovaccine showed highly potent antitumor efficacy in both prophylactic and therapeutic E.G7 tumor models, but simultaneously induced T cell exhaustion by elevating PD-L1 expression, leading to tumor recurrence. After first cycle of immunization, mice were divided into two groups depending on their therapeutic response to the nanovaccine, either good or poor responder group. Only good responder was treated by combination of second immunization cycle of the nanovaccine with anti-PD-1 antibody, resulting in effective inhibition of tumor re-growth. We suggest that our nanovaccine system demonstrated effective antitumor effects and possibility of successful combination immunotherapy with ICB therapy. Especially, combination of these immunotherapies can be rationalized by controlling treatment sequence and timing.

Keywords: Cancer nanovaccine, Immune checkpoint antibody, Cancer immunotherapy, Combination immunotherapy.

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## A074 / Therapeutic targeting of a novel immune modulating receptor leading to a reduction of neutrophil recruitment and a significant reduction of an invasive metastatic proteomic signature

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In this study, a novel receptor for ligands in the TGF beta superfamily, denoted as R2, was investigated as a therapeutic target for solid tumors. The R2 receptor is expressed on the cell surface of immune cells such as neutrophils and macrophages embedded in the tumor micro environment, but also on other subtypes of innate immune cells. In addition, tumor cells can express this receptor. Currently, the R2 receptor is considered to act as a novel modulator of the pro-inflammatory response in the tumor micro environment. In tumor biology it may also take part in chronic inflammatory responses that drive tumor growth.

Herein we have assessed novel antibodies targeting R2 with high affinity. This novel agent is cross-reactive between human and mouse and has been assessed in various tumor models. There was no evident anti-tumor effect when used as a monotherapy in a subcutaneous bladder and breast cancer models, respectively. However, when tumors grew in the lung as a metastases model, regardless of tumor cell origin, there was a notable anti-tumor effect as depicted by reduced numbers of lung tumor nodules post antibody treatment. This effect appeared isotype independent. Interestingly, R2 expression was markedly elevated on neutrophils within lungs of tumor bearing mice compared to neutrophils from the circulation or naïve lungs.

A proteomic profiling was performed on serum collected from mice that were inoculated with MB49 tumor cells 15 days prior and treated i.v. with R2 antibody on days 5, 8 and 11. We noted a significant reduction in systemic levels of Clmp, Eno2, Fst, Hgf and Igsf3 in therapeutically treated animals. The Human Protein Atlas displays that a portion of lung cancer patients, but also other solid cancer patients, have notable mRNA expression elevations of all of these. It is tempting to speculate that the joint expression leads to a niche promoting infiltration of immune cells that can support a metastatic spread of the tumor. HGF has a known effect on neutrophil recruitment by tumors and contributes to immunosuppression and escape from current check-point blockade therapies. Clmp has been suggested to play a role in cell-cell adhesion. Eno2 has been indicated to play a role in bone metastases risk for lung cancer patients. Igsf3 is part of the cytokine receptor signaling transduction indicating that therapy affects chemokine induced cell recruitment. We extracted single cells from lungs of tumor bearing mice and analyzed the immune cell content. Interestingly, both alveolar macrophages as well as neutrophils were significantly reduced post therapeutic intervention. As macrophage and neutrophil infiltration into tumors can counteract the effect of check-point inhibitors by promoting tumor spread and inhibition of T-cells, a combination regimen can be of value for future preclinical and clinical studies.

In summary, we have identified an important novel check-point pathway that, when pharmacologically targeted, relieves the tumor of detrimental neutrophil infiltration and reduces the systemic invasive proteomic profile. Future work will investigate the nature of the response, i.e. if the therapeutic intervention leads to a change in the inflammatory profile of tumor cells or healthy lung tissue, thereby affecting the recruitment of immune cells into the tumor/lung tissue, or if there is a direct antibody-mediated effect that targets immune cells selectively.

Keywords: Receptor for ligands in the TGF beta superfamily, Lung metastasis, Neutrophils.

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## A075 / PRE-CLINICAL DEVELOPMENT OF LISTERIA-BASED NANOVACCINES AS IMMUNOTHERAPIES FOR SOLID TUMORS

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Gold glyconanoparticles loaded with the listeriolysin O peptide 91-99 (Listeria-based nanovaccine), a bacterial peptide with anti-metastatic properties, are vaccine delivery platforms facilitating immune cell targeting and increasing antigen loading. Here, we present proof of concept analyses for the consideration of these nanovaccines as a novel immunotherapy for solid tumors, including cutaneous melanoma. Studies using mouse models of subcutaneous melanoma indicated that Listeria-based nanovaccines recruite and modulate dendritic cell (DC) function within the tumor, alter tumor immunotolerance inducing melanoma-specific cytotoxic T cells, cause complete remission and improve survival. Listeria-based nanovaccines showed superior tumor regression and survival benefits, when combined with anti-PD-1 or anti-CTLA-4 checkpoint inhibitors, resulting in an improvement in the efficacy of these immunotherapies. Sutudies on monocyte-derived DCs from patients with stage IA, IB or IIIB melanoma confirmed the ability of Listeria-based nanovaccines to complement the action of checkpoint inhibitors, by not only reducing the expression of cell death markers on DCs, but also potentiating DC antigen-presentation. We propose that Listeria-based nanovaccines function as immune stimulators and immune effectors and serve as safe cancer therapies, alone or in combination with other immunotherapies, for melanoma and other solid tumors.

Keywords: NANOVACCINES, LISTERIA, IMMUNOTHERAPIES, SOLID TUMORS.

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## A076 / Long-term antitumour immune memory in C57BL/6j mice harbouring GL261 glioblastoma after metronomic Temozolomide administration

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The preclinical GL261 glioblastoma (GB) growing in C57BL/6j mice is an immunocompetent model suitable for assessing immune system participation in therapy response. When using Magnetic Resonance Spectroscopic Imaging (MRSI) coupled to pattern recognition in GL261 GB, 6-7 day periodic metabolome oscillations have been detected [1]. We hypothesized that this could underlie immune system-contributed local changes triggered by response to therapy. Higher CD3 and Iba-1 immunostaining (T-lymphocytes and microglia/macrophages, respectively) was demonstrated in tumour zones categorized as "responding to therapy" in comParison with zones categorized as "unresponsive" (unpublished results). Then, the every 6-days chemotherapy schedule described in [2], now named for clarity: Immune-Enhancing Metronomic Schedule (IMS), was applied to evaluate the impact of such protocol in GL261 GB-bearing mice using Temozolomide (TMZ) to confirm MRSI-detected oscillatory response level and its effect on mice survival.

GB tumours were induced in C57BL/6j mice (n=19) and TMZ administered every 6 days at 60 mg/kg (n=13). T2w Magnetic Resonance Imaging (MRI) and 14 ms TE MRSI were acquired every 2 days. Mice were considered cured (8 out of 13) when the T2 abnormal zone was below 2mm3 during one month. Cured mice (n=8) were followed-up by T2w MRI every 2 days and in case of no tumour mass detected after one month, a "rechallenge" experiment with new GL261 cell injection was carried out, contralateral to the initial injection site, to check out for long-term anti-tumour immune memory, while wt mice (n=3) were also implanted contralaterally as controls. Mice were weighted and inspected twice a week and MRI acquisitions performed once a week.

IMS-TMZ treatment strongly increased survival for GL261 GB bearing mice. IMS-TMZ-treated animals survived 189343.0 days ($p<0.05$), improving standard, non-IMS, TMZ protocol results (33.838.7 days, [3] and unpublished results), while average survival for control GL261 bearing untreated mice was 2131.5 days. MRSI-detected oscillatory response metabolomic pattern was confirmed in seven IMS-TMZ treated animals responding to therapy, provided the tumour volume was suitable for MRSI acquisition and image segmentation. Regarding the re-challenged mice, only one out of eight tumours grew after 10 days, and was again treated with IMS-TMZ, disappearing beyond detection after 17 days post re-implantation (one TMZ cycle). Control GL261 tumours implanted in wt control mice grew normally.

Our findings suggest that antitumour immunological memory is established by the host immune system of cured mice after IMS-TMZ treatment, as described in [2], although additional work will be needed to provide details for the ongoing mechanism. Confirmation of MRSI-sampled oscillating metabolome pattern changes (6.331.4 days after TMZ administration) in GB mice responding to IMS-TMZ treatment suggest that the mice immune system is recruited against GL261 GB, and this can be imaged non-invasively by MRSI. The 6-day interval administration schedule allows us to reduce the cumulative amount of administered TMZ, decreasing the risk of development of other therapy-induced tumours such as lymphomas [4].

Keywords: preclinical glioblastoma, therapy response imaging biomarker, chemotherapy-induced immune response, long-term antitumour immune memory.

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## A077 / Oncolytic Semliki Forest virus shows immunotherapeutic potency in mouse GL261 glioblastoma model

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Glioblastoma (GBM) is a devastating cancer of the central nervous system with no cure currently available. Oncolytic virotherapy with viruses that infect and destroy cancer cells provides a novel promising candidate therapy. Our recent effort has been to develop effective oncolytic Semliki Forest virus (SFV) against GBM. Importantly, SFV's natural capability to home into the central nervous system could allow systemic treatment of brain tumors. SFV induces a robust type-I interferon response in the infected cells. This is beneficial in stimulating innate and adaptive immune responses against the infected cells, but also efficiently inhibits SFV replication and spread in the tumor tissue. By introducing mutations into the viral genome we have been able to produce

novel SFV construct which shows notably enhanced replication in interferon-competent mouse GL261 glioblastoma cells. Robust viral replication was detected despite activated type-I interferon signaling in the infected cells in vitro. This suggests that our new SFV construct has increased resistance to antiviral signaling in mouse GBM cells. The virus was able to infected GL261 tumors ex vivo in brain slice cultures obtained from tumor-bearing mice. SFV proteins could also be detected in intracranial tumors in vivo after intraperitoneal injection of the virus. The SFV-infected areas were also positive for cleaved caspase-3. These results indicate that peripherally administered SFV can reach tumors in the brain and induce oncolysis despite antiviral response in the GBM microenvironment. We found that SFV-infected cancer cells undergo immunogenic apoptosis, which triggers maturation of co-cultured dendritic cells with phagocytosis of apoptotic tumor cells in vitro. Notably, CD11c+ cell could be detected in the SFV-infected/apoptotic regions in vivo, giving evidence of dendritic cell recruitment to the sites of infected/killed tumor cells. Furthermore, vaccination of mice with SFV-killed GL261 mouse glioma lysate was able to induce protective anti-tumor immune response in syngeneic mouse model. This provides further proof that SFV oncolysis is immunogenic and can trigger anti-tumor immunity. Taken together, the results indicate that SFV is a potent new candidate for oncolytic immunotherapy of GBM.

Keywords: glioblastoma, oncolytic virotherapy, alphavirus, Semliki Forest virus.

## A078 / Immunostimulatory transgenes expressed by oncolytic adenoviruses are carried by exosomes released from infected tumor cells – implications for systemic immune activation

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The purpose of this study was to evaluate if the immunostimulatory transgenes expressed in the tumor by oncolytic adenoviruses are spread systemically via exosomes to induce immune activation. Oncolytic viruses are being developed for cancer due to their ability to specifically replicate and induce oncolysis of tumor cells and due to their immune activating capacity. By arming the viruses with immunostimulatory transgenes, the capacity of inducing anti-tumor immunity is greatly enhanced. Exosomes are produced in the endosomal compartment and released as extracellular vesicles. Almost all cell types including cancer cells produce and release exosomes. They can transmit information from one cell to another since they carry nucleic acids, proteins, metabolites etc. In cancer, exosomes have proven importance in the crosstalk between tumor and its stroma but they can also act systemically on distant cells. The exosomes can display proteins in the membrane to directly induce signaling pathways in cells, but exosomes are also taken up by cells which can then be affected by their content. Hence, exosomes can potentially influence the recipient cells through receptor-ligand interaction and reprogramming gene expression. We hypothesized that exosomes released by MEL526 melanoma cells infected by our oncolytic adenoviruses (LOAd) encoding immunostimulatory transgenes cargo the transgenes to affect immune cells, particularly dendritic cells (DCs) at distant sites (1). MEL526 cells were infected with LOAd(-) (no transgenes), LOAd700 (TMZ-CD40L) or LOAd703 (TMZ-CD40L+4-1BBL), or left uninfected, whereupon supernatant was collected for exosome purification. The exosomes, and cell lysates, were analyzed for protein expression via electron microscopy and western blot, and the exosomes were also evaluated for mRNA content by quantitative PCR. The cell lysates from LOAd700- or LOAd703-infected cells contained TMZ-CD40L and TMZ-CD40L + 4-1BBL, respectively. Further, both LOAd700- and LOAd703-infected melanoma cells released exosomes carrying TMZ-CD40L on their surface compared with uninfected cells and LOAd(-)-infected cells. However, we did not find the protein expression of 4-1BBL in the exosomes but the cell line. Protein content was confirmed by western blot. Nevertheless, both TMZ-CD40L and 4-1BBL mRNA were detected in the exosomes released by LOAd703-infected cells while LOAd700-infected cells only carried TMZ-CD40L mRNA as expected. Currently, evaluations are ongoing to investigate the impact of these exosomes on DCs, as well as confirming the presence of transgene cargo in exosomes purified from animals and humans treated with LOAd703.

In conclusion, we demonstrate for the first time that immunostimulatory transgenes expressed by armed oncolytic adenoviruses in tumor cells can be incorporated both as mRNA and expressed protein into tumor exosomes. These exosomes have the potential to act on distant cells to promote anti-tumor immunity and may be an additional mechanism-of-action of armed oncolytic virotherapy.

Keywords: Exosomes, Oncolytic virus, 4-1BBL, TMZ-CD40 ligand.

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## A079 / CD40 antibody therapy is associated with tertiary lymphoid structure formation and impairs the response to checkpoint blockade in glioma

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Glioma is characterized by an immunosuppressive microenvironment that restricts the efficacy of immunotherapy. Here, we demonstrate for the first time that immature tertiary lymphoid structures (TLS) are present in murine models of glioma and that agonistic CD40 antibodies can induce TLS formation in these models. TLS characterization was performed by immunofluorescence and showed that these structures form in proximity of the tumor, close to the meninges. CD40-induced TLS were composed of T cells, B cells, dendritic cells (DCs) and follicular DCs. Interestingly, the presence of immature lymphoid aggregates was observed in en block resected tumors from low grade glioma patients, suggesting that immature TLS are present also in human glioma.

Laser capture microdissection of murine CD40-induced TLS demonstrated an upregulation of TLS-related cytokines such as lymphotoxin-β, CXCL13 and CCL19 within the TLS. Moreover, CD40 stimulation of B cells in vitro and in vivo induced expression of lymphotoxin-α, which is critical for TLS induction. Accordingly, CD40 antibodies were bound to the B cells in the TLS and depletion of B cells previous to anti-CD40 therapy completely prevented TLS formation, suggesting that CD40-activated B cells may act as inducers of these structures.

Following CD40 immunotherapy, the percentage of brain-infiltrating B cells and CD8+ T cells was significantly higher, in accordance with the increased number and area of the TLS. However, a HSNE analysis of multicolor flow cytometry data demonstrated that CD40 therapy was associated with a dysfunctional T cell state characterized by low activation and cytotoxicity, as well as by impaired proliferation. Strikingly, the co-administration of agonistic CD40 antibodies and PD1 blocking antibodies resulted in impaired survival benefit of co-treated mice compared to PD1-treated mice, as PD1 blockade was not able to rescue CD40-induced T cell inactivation.

In summary, this work demonstrates for the first time that agonistic CD40 antibodies can induce the formation of mature TLS in murine models of glioma and that immature TLS-like structures are present in both untreated glioma-bearing mice and low grade glioma patients. Moreover, we demonstrate that combining CD40 stimulation with PD1 blocking antibodies in a repeated co-administration regimen impairs the response to PD1 checkpoint blockade, due to the CD40-induced dysfunctional T cell state. Altogether, our findings describe previously unknown mechanisms of immune regulation in the brain microenvironment that could

aid the development of new immunotherapies for central nervous system malignancies.

Keywords : CD40 immunotherapy, Tertiary lymphoid structures, Ectopic lymphoid structures, Glioma.

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## A080 / The role of killer cell immunoglobulin-like receptors and PD-1+ natural killer cells in resistance to PD-1 blockade in lung cancer

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PD-(L)1–blocking antibodies have clinical activity in metastatic non–small cell lung cancer (NSCLC) and mediate durable tumor remissions. However, the majority of patients are resistant to PD-(L)1 blockade (1, 2). Understanding mechanisms of primary resistance may allow prediction of clinical response and identification of new targetable pathways. Here we present a study where peripheral blood mononuclear cells were collected from 35 patients with NSCLC receiving nivolumab monotherapy and cellular changes, cytokine levels, gene expression, and polymorphisms were compared upon treatment between responders and non-responders. We identified a genetic variant of a killer cell immunoglobulin-like receptor (KIR) KIR3DS1 that is associated with primary resistance to PD-1 blockade in patients with NSCLC. This association could be confirmed in independent cohorts of patients with NSCLC. In a multivariate analysis of the pooled cohort of 135 patients, the progression-free survival was significantly associated with presence of the KIR3DS1 allele (HR, 1.72 ; 95% confidence interval, 1.10–2.68 ; P = 0.017). No relationship was seen in cohorts of patients with NSCLC who did not receive immunotherapy. Cellular assays from patients before and during PD-1 blockade showed that resistance is likely due to NK-cell dysfunctionality. Accordingly, we find that tumor-infiltrating NK cells from NSCLC patients express elevated levels of the immune checkpoint receptors PD-1, TIM-3, and TIGIT on their cell surface. PD-1+ NK cells co-expressed more inhibitory receptors compared to PD-1- NK cells suggesting that reduced effector function of intratumoral NK cells correlates with PD-1 expression on NK cells. Notably, treatment with anti-PD-1 blockade was able to reverse PD-L1 mediated inhibition of PD-1+ NK cells, highlighting the therapeutic potential of PD-1+ NK cells in immune checkpoint blockade. Our findings link NK cells with response to PD-1 therapy in NSCLC and reveal new potential pathways that could be exploited to improve immunotherapy.

Keywords : NK Cells, Checkpoint Blockade, Resistance, KIR.

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## A081 / Target cell-dependent T cell activation by the asymmetric IgG-like bispecific antibody for cancer therapy

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Development Center for Biotechnology (DCB) has developed a novel asymmetric IgG-like bispecific antibody (BsAb) platform for cancer therapy. The BsAb composes the Fc-scFv fusion. In the case of IgG-scFv BsAb, the full length IgG targets the tumor associated antigen (TAA) and the scFv which linked to the C-terminus of one of the heavy chain targets the T cell for engaging and activation. We have demonstrated that the TAAxCD3 BsAb has great potency to eradicate the tumor cells. To minimize the side effect when the BsAb encounters the T cell prior to the target cancer cells, the Fc of the BsAb was engineered to reduce the effector function. In the cytokine secretion study, the TAAxCD3 BsAb with wildtype Fc induces IL-2 production in the absence of target cancer cells, whereas the Fc-engineered TAAxCD3 BsAb induces the cytokines production only in the presence of target cancer cells. This property reduces the safety concern when used in the clinics.

In conclusion, the asymmetric IgG-like BsAb possesses various advantages comparing to other formats, including (1) simple construction without labor intensive screening ; (2) high productivity comparable to its parental mAb ; and (3) target-dependent T cell activation.

Keywords : Bispecific, Antibody, Cancer Therapy.

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## A082 / Macrophage repolarization therapy in ovarian cancer tissue : taking advantage of adipose tissue

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The density of tumor-infiltrating T cells (TILs), most importantly their accumulation inside tumor epithelial islets, is directly related to the overall survival and progression-free survival in epithelial ovarian cancer (EOC) across various histological subtypes. Nonetheless, recent trials in EOC with immune checkpoint blockade remain scarce and have yielded disappointing results. There is currently no approved immunotherapy for this disease, leaving women with a chemotherapy-resistant relapse with no other option than palliative care. Here we investigate the immune landscape and search for targetable factors in metastases that have infiltrated the omentum, which is a frequent complication in EOC. Others have indeed demonstrated the tropism of EOC cells for the adipose tissue of the omentum, which fuels rapid tumor cell proliferation, and the omentum is a preferred site for metastasis in EOC.

Therefore, we used a cohort of EOC specimens that have or have not infiltrated the omentum for a comparative analysis based on histology and multiplex cytokine profiling. We cultured patient-derived explants ex vivo with immunomodulatory drugs and assessed immune cell density, distribution, and activation status.

Tumor sections harboring adipose tissue in close contact with tumor cells are infiltrated by high numbers of cytotoxic TILs, which are metabolically prone to activation but do not infiltrate the tumor core and rather accumulate around adipose tissue. TILs in adipose-rich tumors secrete high amounts of the tumor-supportive chemokine CCL5. These tumors also exhibit a subset of dysfunctional CD163+ macrophages that aberrantly store lipid droplets. The inhibition of the CCL5 receptor CCR5 using the small molecule inhibitor Maraviroc in whole tumor tissue explants leads to the redistribution of cytotoxic TILs towards the tumor core (“hot tumor pattern”), in a CXCL9- and CXCL10-dependent mechanism, but this effect requires the presence of functional macrophages in the tissue. In macrophage cultures, CCR5 inhibition leads to the disappearance of lipid droplets.

Similarly to CCR5 inhibition, the inhibition CD36 – a receptor mediating long chain fatty acid import – alleviates the macrophages from lipid storage in cell culture experiments, which leads to their repolarization into a TNF-α-driven cytotoxic phenotype, similar to the phenotype induced on monocytes by the presence of LPS from bacteria strains isolated from the omentum. Finally, the macrophage-repolarizing effects induced by CD36 blockade on macrophages are also observed in tissue explants, where they are accompanied by a large expansion of cytotoxic TILs. These effects are restricted to tumor explants in which adipose tissue is observed, but not in fat-free tumor samples.

Our results illustrate the dichotomous effects of the presence of adipose tissue close to the tumor on immune cells in omental metastases of EOC, including the protection of T cells from metabolic exhaustion and the maintenance of scavenger macrophages. Tackling the fat storage in these macrophages can effectively turn a "tumor excluded TIL infiltration pattern" into a "hot tumor pattern" by selectively mobilizing cytotoxic T cells around adipose tissue and redistributing them towards the tumor core, in a mechanism that relies on chemokine gradients and on in situ macrophage repolarization into TNFα-driven cytotoxic cells. Finally, the possibility of non-invasive stratification of patients whose tumors have reached the omentum by MRI imaging allows to select patients who could potentially benefit from Maraviroc treatment.

Keywords: ovarian cancer, immunometabolism, adipose tissue, macrophage.

## A083 / The hexavalent CD40 agonist HERA-CD40L induces a productive T cell-mediated anti-tumor immune response and shows superior activity in comParison to benchmark CD40 agonistic antibodies

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TNF Receptor Superfamily members (TNF-R-SF), including CD40, have been key immunotherapeutic targets for over 20 years. CD40 signaling serves as an important co-stimulatory signal for antigen-presenting cells. Along this line, multiple strategies to induce an anti-tumor immune response by promoting CD40 signaling are being investigated. They can be broadly grouped into antibody-based or CD40 ligand (CD40L)-based approaches. Currently, seven different antibodies are in active clinical trials. The limited clinical efficacy of CD40 agonists results from structural and functional characteristics of antibodies, including the presence of only two target-binding domains per molecule, that are unsuitable for stimulating the TNF-R-SF. TNF-R-SF signaling is a structurally well-defined event that requires proper receptor clustering and trimerization. While the ligands naturally exist as trivalent functional units, the receptors are separated on the cell surface and need to be organized into trimeric assemblies. Multiple clusters of trimeric receptor assemblies are necessary to induce proper intracellular domain organization and signaling.

To overcome the inadequacies of bivalent antibodies, we have developed HERA-CD40L. It is composed of two trivalent CD40L-receptor-binding domains, each in a single chain arrangement, fused to a silenced human IgG1 Fc-domain, which serves as a dimerization scaffold. This generates a hexavalent molecule that mimics the natural ligand and enables efficient receptor clustering.

HERA-CD40L treatment increased the pro-inflammatory state of all CD40-expressing cells examined. It promoted the licensing of DC, macrophages, B cells and other APC. We performed extensive comParisons to multiple benchmark antibodies in development. Significant differences in CD40 signaling were visible immediately following treatment. For example, HERA-CD40L elicited stronger and more rapid activation of NFkB signaling in primary B cells. In addition, HERA-CD40L, but not clinical benchmark, triggered immediate NFkB, MAPK, PI3K and STAT-1 signaling in primary monocyte-derived immature DC, as shown by phospho-specific flow cytometry. As a result, HERA-CD40L induced stronger upregulation of activation and antigen presentation markers, including co-stimulatory molecules, in B cells and DC. Importantly, using SEC-purified material in a cellular CD40 reporter assay, the activity of the clinical benchmark antibody almost exclusively derived from antibody aggregates, resulting in a more than 1000-fold drop in activity compared to the purified monomeric fraction. HERA-CD40L treatment converted immature phagocytic macrophages into mature/professional APC and induced repolarization of M2- to M1-like macrophages, in turn promoting potent allogeneic anti-tumor T cell responses that were not detectable with anti-CD40 antibody treatment. The anti-tumor efficacy of the mouse surrogate (mmHERA-CD40L) was confirmed in two different mouse models. In the CT26wt model, mmHERA-CD40L treatment converted cold into hot tumors by increasing CD4+ and CD8+ T cell infiltration. In addition, mmHERA-CD40L showed single agent anti-tumor activity in the MC38-CEA model. Both in vitro (human) and in vivo (mouse), HERA-CD40L increased antigen-specific immune system activation without affecting the non-specific immune cells. These data, together with pilot PD/safety results, demonstrate that the activity of HERA-CD40L is both potent and safe. In summary, HERA-CD40L shows single agent anti-tumor activity both in vitro and in vivo. The biological activity is distinct from and superior to clinical benchmark "agonistic" antibodies. HERA-CD40L has a well-defined mechanism of action, does not depend on Fc gamma receptor-mediated cross-linking and hence functions as a true agonist.

Keywords: CD40, CD40L, TNF agonist, TNF superfamily, Hexavalent receptor agonist (HERA).

## A084 / The oncolytic adenovirus, LOAd703 induces immune cell activation and controls tumor cell growth in multiple myeloma models

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In this study, we aimed to evaluate the oncolytic and immunostimulatory capacity of oncolytic adenoviruses serotype 5/35 stimulating the 4-1BB and/or CD40 pathway in models of multiple myeloma (MM). MM is a malignant disease with extensive morbidity and mortality characterized by the abnormal growth and accumulation of malignant plasma cells in the bone marrow. Although MM treatment has improved substantially over the past decades, MM still remains incurable as most patients develop resistance to current treatments and relapse. MM has been associated with high degree of immune dysregulation which results in evasion of immune surveillance. Thus, utilizing oncolytic viruses armed with immunostimulatory transgenes may restore anti-tumor immunity at the same time inducing tumor cell oncolysis. Our group has developed a new platform of oncolytic serotype 5 adenoviruses which conditionally replicates in tumor cells due to a deletion in E1A and infects CD46+ cells due to a serotype 35 fiber (LOAd). Further, LOAd viruses encodes different immunostimulatory genes, where LOAd700 encodes for trimerized membrane-bound (TMZ) CD40L and LOAd703 encodes TMZ-CD40L as well as 4-1BBL. LOAd(-) lacks transgenes. Deletion of Retinoblastoma (Rb), CD46 overexpression and deficiencies in IFN signaling make MM cells more sensitive to viral infection and thereby a good target for LOAds. Herein we investigated the oncolytic and immunostimulatory capacity of LOAds in a panel of MM cell lines (ANBL-6, L363, LP-1, OPM-2, RPMI-8226, and U266-84). The LOAd virus-mediated oncolysis of MM cells were analyzed by MTS assay and virus replication was confirmed by qPCR detecting adenoviral DNA. Transgene expression and immune markers were analyzed by flow cytometry. Cell culture supernatants were investigated by multiplex analysis. Additionally, LOAd proficiency in stimulating immune cells activation was evaluated by co-culturing MM cells with peripheral blood mononuclear cells (PBMCs). For in vivo experiments a xenograft model of BALB/c nude mice injected with subcutaneously RPMI8226 was utilized. LOAd viruses effectively killed all MM cell lines tested and replication was confirmed already after 24 hours post infection. The transgenes, TMZ-CD40L and 4-1BBL, were expressed in all cells infected with the respective viruses carrying the transgenes. Interestingly, the apoptosis receptor Fas was upregulated post LOAd infection in

all cell lines. The pro-inflammatory cytokine MIP-1α was increased in 4/6 cell lines and the MM growth factor MCP-1 as well as sIL2R were decreased in 3/6 cell lines. However, CD70 and ICAM-1 were decreased post infection in five of six cell lines. In the co-culture of MM with PBMCs, IFNγ levels as well as activation markers including CD69 and CD107a were upregulated in cultures infected with LOAds. The best effect was seen after LOAd703 infection. In the xenograft model, intratumoral administration of LOAd703 controlled the tumor growth and extended the overall survival where intravenous administration had a lesser effect.

In conclusion, LOAd infection not only upregulates markers associated with immune cell activation such as CD69 and CD107a, but also induce a more immunogenic tumor by increasing the expression levels of Fas receptor (important for triggering programmed cell death cascade) and downregulating markers associated with tumor growth and survival. Hence, LOAd viruses such as LOAd703 may be a promising therapeutic approach for treating MM.

Keywords: Multiple myeloma, Oncolytic virus, CD40L, 4-1BBL.

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## A085 / Molecular retargeting of antiviral antibodies to tumor cells by using bispecific adapter proteins inhibits tumor growth in mice

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Treatment of solid tumors using oncolytic adenoviruses is a promising approach to achieve selective tumor cell killing, reduction of tumor immunosuppression and induction of anti-tumor immune responses. However, this treatment also triggers strong humoral immune responses against the adenoviral vector, mainly against the virus capsid, thus limiting viral spread and efficacy of repeated administrations. Here, we investigated whether these anti-adenoviral antibodies can be converted into a cancer immunotherapeutic tool by means of tumor-specific molecular retargeting. We established bispecific adapter molecules harboring a domain derived from the adenoviral capsid protein hexon, which is capable of binding substantial amounts of anti-adenoviral IgGs. The adapter molecules furthermore contained a tumor-specific ligand such as a single chain variable fragment recognizing polysialic acid (polySia) which is overexpressed on the cell surface of tumor cells in several clinically relevant cancers including glioblastoma and lung cancer. In adenovirus pre-challenged mice, systemic application of this adapter molecules inhibited tumor growth and significantly prolonged survival in several syngeneic, subcutaneous tumor models established with CMT64 lung carcinoma, MC38 colon carcinoma, and B16F10 melanoma cells as well as in a transgenic tumor model of orthotopic, polySia expressing liver cancer. Antibody retargeting effectively inhibited metastases in a murine model of CMT64 lung colonies established by intravenous tumor cell instillation. Analyses of tumor infiltrating cells and subsequent depletion experiments revealed NK-cells and CD8 T-cells as essential mediators of adapter-mediated tumor growth inhibition. Furthermore, we could show that adapter treatment leads to a systemic induction of tumor antigen-specific CD8 T-cells in a NK cell-dependent manner indicating that NK cell-mediated tumor cell killing is an important step for priming of tumor-directed CD8 T cells. Antibody retargeting following an intratumor treatment of MC38 tumors with an oncolytic adenovirus resulted in enhanced therapeutic efficacy compared to virotherapy alone, including complete tumor remissions and long-term tumor free survival. Finally, we demonstrated that antibody retargeting immunoactivated tumors to act synergistically with PD-1 checkpoint inhibition. A rational therapeutic scheme consisting of intratumor virotherapy, subsequent antibody retargeting and PD-1 checkpoint inhibition led to survival in the majority of treated mice. Our data demonstrate that retargeting of antiviral antibodies using bispecific adapter molecules is a promising new approach for cancer immunotherapy and significantly improves the therapeutic potential of an oncolytic virotherapy.

Keywords: antiviral antibodies, oncolytic virus, cancer immunotherapy.

## A086 / Interleukin-33 is a novel activator of a subset of polyfunctional NK cells with anti-tumor activity

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Natural Killer cells have the potential to eliminate tumors through their ability to directly recognize and kill cancer cells but also through their production of cytokines which are important to elicit and support an effective anti-tumor immune response. However, NK cells infiltrating tumors show impaired effector functions and their response to classical stimuli seems altered. Understanding mechanisms suppressing NK cells activity in tumor microenvironment and identifying new candidates for NK cell activation is therefore of great interest to develop new therapeutic strategies.

In this context, we are evaluating the potential of Interleukin-33 (IL-33) as a novel activator of NK cells, in physiological and pathological context of breast cancer (BC). IL-33, considered as an alarmin released from the nucleus of non-immune cells upon cellular stress or damage, is a member of the IL-1 family and binds the receptor ST2 to promote both type 1 and type 2 immune responses. Consistently, paradoxical roles of IL-33 were reported, promoting either anti-tumoral or pro-tumoral immune response. These observations prompted us to investigate IL-33/ST2 signaling in NK cells from healthy donors (HDs) and BC patients. We unraveled a new pathway for NK cells activation where IL-12 up-regulates ST2 in a STAT-4-dependent signaling pathway on a subset of human blood NK cells which are CD56dim CD16+ CD57-/+. Following IL-33 stimulation, ST2+ NK cells secrete high levels of pro-inflammatory cytokines (IFN-γ, TNF-α, XCL1, MIP1α and MIP1β), increase their cytotoxic activity, and retain high proliferative ability. In parallel, in the context of human BC, we observed that a subpopulation of tumor-infiltrating NK cells express ST2 at steady state. Interestingly, approximately 20% of tumor-infiltrating NK cells and blood NK cells from BC patients respond to IL-33 when combined with IL-12, supporting a good potential for the use of IL-33 to re-activate NK cells' anti-tumor immune response. We are currently evaluating the role of endogenous and exogenous IL-33 on tumor progression in vivo using a mouse model of triple negative breast cancer in WT mice vs il33-KO mice.

ST2+ NK cells therefore represent an unprecedentedly characterized intermediate subtype between canonical CD56bright and CD56dim NK cells showing phenotypic markers and functions of both subtypes. As different epigenetic programs define NK cells differentiation from CD56bright to CD56dim, our hypothesis is that ST2+ NK cells possess a unique chromatin landscape allowing ST2 expression and therefore NK cells' ability to respond to IL-33. Ongoing ATAC-seq and RNA-seq analysis will help us to decipher the main molecular characteristics of ST2+ versus ST2- human NK cells.

Keywords: NK cells, Immunotherapy, Breast Cancer, Interleukin-33.

## A087 / Cancer immunotherapy with oncolytic virus for the treatment of peritoneal carcinomatosis of colon cancer

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Peritoneal carcinomatosis is a common but devastating form of metastases in colon cancers and remains as a difficult clinical challenge because it is usually refractory to conventional therapies. Here, we explored the therapeutic potential of oncolytic vaccinia virus in the treatment of peritoneal carcinomatosis of colon cancer. MC38 colon cancer cells were intraperitoneally injected to C57Bl6/ mice to generate peritoneal carcinomatosis. Tumor-bearing mice were treated with either oncolytic vaccinia virus or anti-PD-1 antibody every three days. Tumor size, the volume of ascites, and peritoneum seedings were monitored after the treatment. The tumor microenvironment was comprehensively analyzed with multiplex immunofluorescence imaging, flow cytometry, and Nanostring analysis. Oncolytic vaccinia virus infected, replicated, and destroyed the tumor cells of peritoneal seeding nodules of colon cancer. Moreover, oncolytic virus treatment significantly delayed the progression of peritoneal seeding nodules of colon cancer and reduced the volume of malignant ascites. It also increased the intratumoral infiltration of CD8+ T cells and induced a strong tumor-specific anti-tumor immune response within tumors and lymphoid organs. Nanostring analyses of whole tumor tissues revealed dramatic changes of immune-related gene signature after oncolytic virus treatment. The combination immunotherapy of oncolytic virus and anti-PD1 antibody elicited greater anti-tumor immunity than monotherapy and prolonged the survival of tumor-bearing mice.

In conclusion, oncolytic vaccinia virus effectively suppressed the peritoneal carcinomatosis of colon cancer, reduced the formation of malignant ascites, and synergized with PD-1 immune checkpoint to enhance anti-tumor immunity against colon cancer.

Keywords: Oncolytic virus, Peritoneal carcinomatosis, Colon cancer, Combination Immunotherapy.

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## A088 / Immobilized synthetic polymers as a novel cancer immunotherapy to activate and expand tumor-reactive T cells

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Traditional tumor vaccination approaches mostly focus on activating dendritic cells (DCs) by providing them with a source of tumor antigens and/or adjuvants, which in turn activate tumor-reactive T cells. This strategy, however, is highly dependent on DC maturation and context, resulting in poor control over what stimulatory signals are provided to T cells. Novel biomaterial-based cancer immunotherapeutic strategies focus on directly activating and stimulating T cells through molecular cues presented on synthetic constructs with the aim to improve T cell survival, more precisely steer T cell activation and direct T cell differentiation. Artificial antigen-presenting cells (aAPCs) decorated with T cell-activating ligands are being developed to induce robust tumor-specific T cell responses, essentially bypassing DCs. Filamentous polyisocyanopeptide polymers (PICs) are highly promising synthetic aAPCs that display unique semiflexibility and strain-stiffening behavior. PIC polymers (200–400 nm in size) can be functionalized with multiple biomolecules such as antibodies and cytokines that can be presented to T cells in a multivalent manner to evoke robust T cell responses (1-3). Here, we developed a novel system which enables site-specific attachment of end-functional PIC polymers to a surface (4). This system allows for immobilization of PIC polymers on (magnetic) beads or plate surfaces to create a unique platform to deliver the activating cues to T cells on a semiflexible brush. The PIC-modified particles induced higher proliferation and especially improved cytokine production by T cells in vitro when compared to beads directly modified with antibodies or commercially available Dynabeads. Moreover, fewer activating cues were needed to induce T cell activation when presented to the T cells via the semiflexible brush surface. In addition, this system is highly modular and allows for attachment of a wide range of biomolecules, which can for instance be used to expand antigen-specific T cells using peptide:MHC complexes. The Introduction of cargo-loaded particles into the system also allows for the delivery of small molecules, such as cytokines or TGF-beta inhibitors , to T cells. All in all, this approach is widely applicable and holds great promise for both in vitro and in vivo cancer immune T cell-therapies.

Keywords: synthetic antigen-presenting cells, T cells, Biomaterials, molecular cues.

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## A090 / Serial antibody responses to XAGE1 and NY-ESO-1 antigens during anti-PD-1 therapy for non-small-cell lung cancer

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Background: Cancer-testis (CT) antigens such as NY-ESO-1 are expressed broadly in various types of human cancers, and the antigens elicit spontaneous cellular and humoral immune responses. XAGE1 CT antigen among them is frequently expressed in lung adenocarcinomas, and serum antibodies (Abs) against XAGE1 and NY-ESO-1 were detected in approximately 25% of advanced non-small-cell lung cancer (NSCLC) patients, as we reported previously. On the other hand, programmed cell death-1 (PD-1) inhibitors effectively treat NSCLC and prolong survival, resulting in a standard therapy for advanced NSCLC. We previously reported that XAGE1 and NY-ESO-1 serum Abs predicted clinical benefits of good responses and long survival with anti-PD-1 monotherapy for NSCLC and that the Ab titers strongly correlated with tumor reduction rates. In this study, XAGE1 and NY-ESO-1 serum Abs were serially measured to investigate the relationship between clinical responses and immunological responses to the antigens after anti-PD-1 monotherapy for NSCLC.

Materials and Methods: Sera were serially obtained from NSCLC patients before and after anti-PD-1 therapy (nivolumab or pembrolizumab), and serum Abs against XAGE1, NY-ESO-1, and p53 protein were measured by ELISA. Concomitantly, Ab responses to other tumor antigens were also examined in 27 NSCLC patients, using a multiplex Luminex beads assay with a panel of 50 recombinant tumor antigens. Fifty antigens consisted of 38 CT and 11 overexpressed-protein antigens and one other SOX antigen. The O.D. values of Ab responses against each tumor antigen were converted into a score from 0 to 100. A positive Ab response was defined as that exceeding or being equal to a score of 75 (≥75).

In addition, an "increased Ab response" against each antigen was defined using the following formula (score after therapy - score before therapy = difference ≥25), indicating a newly recognized antigen after anti-PD-1 therapy. In order to evaluate "antigen spreading" after anti-PD-1 therapy, Ab responses (scores) to 50 tumor antigens were compared before and after anti-PD-1 therapy, and the number of "increased Ab responses" (newly recognized antigens) was counted in each patient. In this study, "antigen spreading" was defined as a newly recognized antigen after anti-PD-1 therapy.

Results: Before anti-PD-1 therapy, XAGE1 and/or NY-ESO-1 serum Abs were positive in nine (33%) out of 27 patients and negative in 18 (67%), and p53 serum Ab was positive in only one patient. Four patients were respectively positive for XAGE1 and NY-ESO-1, and one was positive for both. Six (67%) of nine XAGE1/NY-ESO-1 Ab-positive patients and four (22%) of 18 the Ab-negatives had positive Ab responses to more than two tumor antigens among 50 antigens.

After anti-PD-1 therapy, XAGE1/NY-ESO-1 Ab titers in clinical responders increased within two months and then gradually decreased with tumor shrinkage, and the titers in non-responders were gradually increasing. In addition, the titers in patients with complete response decreased to undetectable levels. Before and after anti-PD-1 therapy, the number of positive Ab responses (score ≥75) was increased in five out of nine XAGE1/NY-ESO-1 Ab-positive patients, remained unchanged in one, and decreased in three, and also remained unchanged in seven out of 12 XAGE1/NY-ESO-1 Ab-negative patients and decreased in five. Furthermore, six (67%) out of nine XAGE1/NY-ESO-1 Ab-positive patients and three (25%) out of 12 XAGE1/NY-ESO-1 Ab-negative patients showed antigen spreading after anti-PD-1 therapy.

Conclusion Our results suggest that NY-ESO-1 and/or XAGE1 serum Abs are useful biomarkers monitoring tumor burden or clinical responses to anti-PD-1 therapy for NSCLC and that the Abs are surrogate markers predicting antigen spreading after anti-PD-1 therapy.

Keywords: Biomarker, PD-1 inhibitor, Lung cancer, Cancer antigen.

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## A091 / ONCR-177, an oncolytic HSV designed to safely and potently activate systemic anti-tumor immunity

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ONCR-177 is a highly modified recombinant oncolytic Herpes Simplex Virus (oHSV) designed to be a safe and efficacious therapy for the treatment of solid tumor indications. ONCR-177 expresses five transgenes selected using an in vivo screen: IL-12 for NK and T cell activation, CCL4, and a secreted FLT3LG for expansion and recruitment of classical dendritic cells (cDC), and antagonists to two clinically validated immune checkpoint targets, PD-1 and CTLA-4, to counter T cell exhaustion. ONCR-177, or its mouse surrogate virus mONCR-171, was tested in a series of experiments designed to characterize its effect on local and systemic anti-tumor activity, its mechanisms of action, and biodistribution. In in vitro co-culture studies, ONCR-177 oncolysates were superior in stimulating MART1+ tumor antigen specific T cells compared to lysates from the base vector (no payloads) ONCR-159. Intra-tumoral administration of mONCR-171 resulted in durable local and distant complete tumor regressions and survival benefit across several syngeneic bilateral tumor models representing various degrees of HSV permissivity and baseline T cell infiltration, including models reported to be resistant to other oHSV therapies. Abscopal anti-tumor activity could not be explained by systemic propagation of the virus since biodistribution studies verified that viral DNA and payload expression were mostly detectable in the injected tumor, with little to none detected in the contralateral tumor, blood, or liver. Importantly, there was no indication of cytokine release syndrome even at high IT doses of mONCR-171, as indicated by low serum IL-6 or TNF-a levels. However, mONCR-171 dose-dependent increases in plasma IFN-gamma and CXCL10 were noted and believed to be on-mechanism and indicative of a productive systemic anti-tumor immune response. Immune phenotyping by flow cytometry of injected or contralateral MC38 syngeneic tumors 8 days after a ONCR-171 IT treatment regimen demonstrated increased numbers of activated NK cells, CD8 and CD4 T cells, and classical dendritic cells. The proportion of Tregs decreased, resulting in large CD8/Treg ratios. These changes in immune contexture occurred in both the injected and contralateral tumor and were more pronounced with mONCR-171 treatment compared to the base vector ONCR-159. Transcriptional profiling yielded similar results as flow cytometry with elevated transcripts indicative of enhanced immune processes such as cytotoxicity, IFN signaling, and antigen presentation. Immune depletion studies demonstrated that CD8 T cells and NK cells are essential for mONCR-171 abscopal efficacy. Re-challenge studies in the A20 and CT-26 tumor models demonstrated that curative mONCR-171 therapy resulted in long-lasting protective immunity. Pre-exposure to HSV-1 and the presence of circulating anti-HSV-1 antibodies did not alter the anti-tumor efficacy of mONCR-171.

In conclusion, mONCR-177/171 potently activates local and systemic anti-tumor immune responses that results in significant tumor cures and extended survival. These encouraging preclinical data warrant the clinical investigation of ONCR-177 in patients with metastatic cancer.

Keywords: Oncolytic virus, miR attenuation, payloads, anti-tumor immune response.

## A092 / Intratumoral application of the RNA-based TLR-7/-8 and RIG-I agonist CV8102 leads to a pro-inflammatory tumor microenvironment and durable anti-tumor effects

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New therapeutic strategies to treat cancer by breaking the cancer-induced immune tolerance rather than targeting the tumor itself have garnered considerable interest in recent years. These therapeutic strategies involve approaches such as activation of immune cells via cytokines, re-activating T cells via immune checkpoint blockade or influencing the tumor microenvironment towards immune activation. Unfortunately, systemic administration of such drugs is frequently accompanied by toxic side effects. We thus sought to leverage local drug administration as an alternative to achieve high drug concentrations in the tumor microenvironment while avoiding the dose-limiting toxicity associated with systemic application. In particular, immune-modulating substances administered intratumorally offer an attractive option to induce a local pro-inflammatory tumor microenvironment and anti-tumoral effects via induction of a systemic immune response. Here, we present a novel approach for the therapy of solid tumors by intratumoral treatment with the RNA-based immune stimulator CV8102.

CV8102 consists of a synthetic non-capped, non-coding, immunostimulatory RNA that is complexed with a polymeric carrier and activates TLR-7/-8 and RIG-I. Previous studies have demonstrated that intradermal injection of CV8102 leads to immune cell activation at the injection site and in the draining lymph nodes. As anti-tumoral responses are often hampered by the immunosuppressive microenvironment of the tumor, we reasoned that intratumoral injection of CV8102 could shift the tumor microenvironment to a more pro-inflammatory state and thereby stimulate an anti-tumoral response. This response might be further enhanced by systemic anti-PD-1 treatment.

To assess the influence of CV8102 on the tumor microenvironment, mice were injected subcutaneously with the syngeneic colon carcinoma cell line CT26 and the established tumors were

injected twice with CV8102 alone or in combination with systemic anti-PD-1 treatment. At 5h after the second injection the gene expression profile of the tumor was analyzed.

Tumors treated with CV8102 alone or in combination with anti-PD1 treatment showed a significant enrichment of genes whose expression is correlated with innate immunity (TLRs, RIG-I, INF-I, IFNγ, CXCL10, CXCL11, IL15, IL18) and response to viruses. Importantly, CV8102 strongly increased expression of interferon stimulated gene (ISG) that are known to be critical not only for immune recognition of tumor associated antigens and immune cell recruitment but also for tumor destruction. In line with the effect of CV8102 on ISGs, our data showed that CV8102 synergizes with anti-PD1 treatment to significantly enrich genes whose expression is correlated with adaptive immunity and cytotoxicity. In contrast, systemic anti-PD-1 treatment alone did not induce any of the mentioned pathways in the tumor.

The increased immune activation in the tumor was reflected by a substantially reduced tumor growth in CT26 tumor-bearing mice that were treated twice a week for three weeks with CV8102. The anti-tumoral effect of CV8102 was dose-dependent and induced complete remission in up to 50% of treated mice. In combination with systemic PD1 immune checkpoint inhibition even low doses of CV8102 administered intratumorally resulted in complete tumor eradication in the majority of mice.

Based on these promising preclinical data, a Phase I clinical trial is currently ongoing to assess the safety and tolerability of intratumoral application of CV8102 in combination with immune checkpoint blockade. Preliminary data show that intratumoral CV8102 appears to be well tolerated. Importantly, evidence of clinical single agent activity with shrinkage of injected and non-injected lesions has been observed.

Keywords : intratumoral therapy, innate immune stimulation, RNA, TLR/RIG-I Agonist.

## A093 / Targeting ICOS/ICOSL pathway for enhancement of cancer immunotherapy

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Immunotherapy has revolutionized the treatment for different types of cancer. Currently, immune checkpoint drugs, Ipilimumab, Nivolumab, and Pembrolizumab, have shown remarkable clinical responses in a broad range of advanced-stage malignancies. In addition to checkpoint blockade, a number of studies have shown that immune co-stimulators also as crucial players in the immunotherapy. Upon TCR stimulation, inducible co-stimulator (ICOS) is induced to express on activating T cells. Through binding to its ligand, ICOSL, ICOS-mediated signaling can further enhance T cells proliferation and activation. Previous studies have also shown that, ICOS/ICOSL pathway may play an important role in CTLA4 inhibitor-induced tumor immunity. In this study, we aim to develop and implement the Fc-fusion recombinant ICOSL proteins (DCB-IM) to increase T cell activation for therapeutic use. Our data showed that DCB-IM enhanced the proliferation and cytotoxicity effect of human T cells through increasing INFg and IL10 secretion. Moreover, combination treatment of DCB-IM and anti-CTLA4 antibody significantly inhibited tumor growth in xenograft animal models. Basing on our study, ICOS/ICOSL pathway could be a promising therapeutic target for the immunotherapy.

Keywords : Immunotherapy, ICOS, ICOSL.

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## A094 / Synthetic glycolipid-peptide vaccines directed against human HER2 prevent lung metastasis of breast cancer

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Lung metastasis can complicate many cancers, including breast cancer, and carries a poor prognosis. We employed a model of human HER2+ breast cancer to determine whether invariant natural killer T (iNKT) cell-activating vaccines could generate protective T-cell responses in the lung against this clinically-relevant human oncogene. We designed vaccines (α-GalCer-L-HER263-71) comprising the HLA-A24- and H-2Kd-restricted human HER2 epitope TYLPTNASL (HER263-71) chemically conjugated via a cathepsin cleavable linker to the glycolipid α-galactosylceramide (α-GalCer). Following intravenous administration of α-GalCer-L-HER263-71 to BALB/c mice, we observed strong activation of iNKT cells and antigen presenting cells (APCs) at 18 hours. We found a population of HER2-specific CD8+ T cells in the lung by tetramer staining and cytotoxic activity was observed against syngeneic peptide-pulsed targets using an in vivo cytotoxicity assay. Finally, vaccination with α-GalCer-L-HER263-71 prevented lung colonisation of intravenously-administered 4T1.2-HER2 tumor cells. These results suggest that iNKT cell-activating conjugate vaccines may have utility for the prevention of metastasis of HER2+ cancers, and provide proof of principle that this class of vaccine can protect against clinically-relevant tumor antigens.

Keywords : Breast cancer, Synthetic Vaccine, iNKT cells, HER2.

## A095 / Collapse of the plasmacytoid dendritic cell compartment in metastatic melanoma by the tumor secretome and lactic acidosis.

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Among immune cells involved in cancer immunity, plasmacytoid dendritic cells (pDCs) exert an important role bridging the innate and adaptive immune responses. A distinctive feature of pDCs is the production of high amount of type I Interferon, through the Toll-like receptor (TLR) 7 and 9 signaling pathway activation. In cutaneous melanoma, the pDCs recruitment is recurrent in primary lesions, but limited data are available on the dynamic of the pDC compartment over melanoma progression. In metastatic melanoma (MM) patients, the prognosis has remained poor for a long time. However, the recent Introduction of effective target therapies (BRAF and MEK inhibitors) and immunotherapies (anti-CTLA-4 and anti-PD-1) has significantly improved the survival of MM patients. Of note, these responses are highly dependent on the fitness of the host immune system, including the innate compartment. Our leading assumption is that a proper activation of the pDC compartment might represent a clinically relevant adjuvant. Here we provide a set of data showing that melanoma cells-dependent lactic acidosis support the collapse of the pDC compartment. The frequency of pDCs was evaluated in a cohort of 29 MM patients and 25 healthy donors (HD) by flow cytometry on whole blood samples. Sixteen MM patients were BRAF mutated, seven were NRAS mutated and six were BRAF/NRAS wild-type. Among BRAF mutated MM patients, four received BRAFi and twelve received the combination of BRAFi and MEKi. Blood samples were obtained at different time points over treatment, based on the expected time to the clinical response to therapy. For in vitro experiments, peripheral blood pDCs were magnetically sorted from buffy coats of HD and subsequently exposed to melanoma cell lines supernatants (SN-mel) or to increasing concentration of lactic acid, sodium lactate and hydrochloric acid.

pDCs were also treated with BRAFi alone or in combination with MEKi. The pDCs viability was evaluated by flow cytometry.

Circulating pDCs are severely reduced in chemotherapy-naïve MM patients compared with HD, particularly in the M1c group characterized by an increased LDH level and the worst prognosis. No differences were observed based on the molecular profile of the tumors. The significant reduction of blood pDC compartment could be explained by an unfavorable interaction of pDCs with melanoma cells or tumor secretome. We found that in vitro exposure to SN-mel affects pDCs viability and activates the apoptotic pathway. Furthermore, soluble components of the melanoma secretome, but not extracellular vesicles, affect the viability of pDCs. By the analysis of a prospective MM cohort of chemotherapy-naïve patients, we found that the pDCs frequency is inversely correlated with the overall survival (OS) of chemotherapy-naïve patients. Moreover, no recovery of the blood pDCs could be observed during the treatment in BRAF mutated MM patients, although BRAFi plus MEKi could slightly reduce the pDCs viability. Notably, a high serum level of LDH and a high tumor burden are associated with a significantly reduced frequency of pDCs. Accordingly, the viability of pDCs was significantly impaired by in vitro induced lactic acidosis, suggesting a role for LDH in the collapse of the differentiated pDC compartment.

The present study demonstrate that the circulating pDC compartment is collapsed in MM and no recovery could be observed in BRAFi/MEKi treated MM patients. Significantly, our in vitro findings indicate that components released by melanoma cells as well as systemic and local lactic acidosis might exert a relevant effect on the survival of fully differentiated pDCs.

Keywords: plasmacytoid dendritic cells, melanoma.

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Vescovi R, et al. Cancer Immunol Res. 2019 Jan;7(1):12-28.

## A096 / Identification of peripheral blood CD8+ T cell subsets correlated with response to PD-1 Ab in melanoma patients by single cell gene expression analysis

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Immune checkpoint inhibitors (ICIs) have been approved for patients with various advanced cancers, but responses are still limited. Thus, identification of biomarkers for prediction of PD-1/PD-L1 Ab response particularly using peripheral blood samples is required. In this study, we evaluated peripheral blood CD8+ T cell subsets by using single cell RNA-sequencing (scRNA-seq) technique, which may be correlated with responses to the PD-1 Ab, nivolumab in patients with metastatic melanoma. scRNA-seq analysis of CD8+ T cells was performed in 9 Japanese melanoma patients (PD4, PR3, long-SD2). Single CD8+ T cells were sorted from peripheral blood using a flow cytometer. cDNA was synthesized from CD3+CD8+PI- gated single cells using a SMART-Seq v4 3' DE kit (Clontech) for the generation of a 3' library preparation followed by next-generation sequencing performed by the NovaSeq6000 sequencer. 3, 456 sorted cells were subjected to in silico analysis, and 3, 206 cells were passed by quality check, resulting in an average of 2.7 million mapped reads and ~6, 000 genes detected per cell. Gene clustering was performed by using the Seurat R package. Monocle trajectory analysis was also performed to map potential developmental trajectory of CD8+ T cell subsets, showing the cell phenotype transition from naïve to effector cells. Based on t-distributed stochastic neighbor embedding (t-SNE) analyses, we identified 8 clusters in CD8+ T cells. Among these 8 CD8+ T cell clusters, a cluster enriched for stem cell memory-like feature highly expressing TCF7 gene was higher at both baseline (p=0.03) and 7 weeks following PD-1 Ab nivolumab treatment (p=0.056) in responders (PR or long SD) compared to non-responders. This stem cell memory-like CD8+ T cell cluster was increased in 4 of 5 responders at 7 weeks following nivolumab treatment. In addition, a GZMK-positive pre-exhausted CD8+ T cell cluster was increased in 1 of 5 responders. This memory-like CD8+ T cell subset had relatively low expression of the cytotoxic genes, PRF1 and GZMA. High TCF7+ CD8+ T cells in tumors have previously been reported to be correlated with favorable response to PD-1 Ab in melanoma. In this study, we found the increase of similar CD8+ T cell subsets in peripheral blood was also correlated with favorable response to PD-1 Ab. These results indicate that particular CD8+ T cell subsets identified by scRNA-seq analyses in peripheral blood may lead to new biomarkers for prediction of PD-1 Ab response in melanoma patients.

Keywords: Melanoma, Nivolumab, scRNA-seq.

## A097 / A 3D organoid and T cell co-culture model to study mechanisms of immunotherapy response and resistance in vitro

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Most colorectal cancers (CRCs) do not respond to immunotherapy with checkpoint blocking antibodies. Modest mutation loads and immunosuppressive microenvironments, including high TGFβ expression and infiltration with regulatory T cells (Tregs) and macrophages likely contribute to this (1, 2). The CEA-TCB bispecific antibody is a novel therapeutic agent that can help overcome the unfavourable immune landscape in CRC. CEA-TCB binds CD3 on T cells and targets them to the carcinoembryonic antigen (CEA) which is overexpressed on the cell surface of many CRC cells (3, 4). Recent Phase I clinical trial data in patients with CEA positive CRCs showed that not all patients responded to this treatment (5). In order to understand the molecular mechanisms determining resistance and sensitivity to such immunotherapies, there is a major need to develop laboratory models that enable detailed studies of immune and cancer cell interactions facilitated by bispecific antibodies. We developed an immunocompetent CRC in vitro model that recapitulates key features of colorectal tumors which are absent in other preclinical models. Cancer cells derived from patients with advanced and drug resistant CRC are grown as 3D spheroids (so called 'patient derived organoids', PDOs) in a matrigel-based extracellular matrix. This maintains cell-cell and cell-matrix interactions as well as spatial structures which likely generate physical barriers and chemical gradients. Combination with allogeneic CD8 T cells and labelling of both cell types (with nuclear GFP and a live-cell tracking dye) allows live confocal microscopy imaging over 3 days. We show that T cells readily infiltrate into cancer cell spheroids and kill CRC cells when CEA-TCB is added. Distinct PDO morphologies such as growth as compact or hollow spheres did not impair T cell infiltration. However, we revealed heterogeneity of T cell infiltrates between morphologically similar cancer cell spheres within the same PDO line. We are now increasing the complexity of this 3D immunogenic model by incorporating additional cell types and cytokines that can be abundant in the CRC microenvironment such as cancer associated fibroblasts, macrophages, and Tregs. Monitoring T cell migration, infiltration into and killing of PDOs in the presence and absence of CEA-TCB should define novel determinants of CEA-TCB activity. Updated data will be presented.

Keywords: in vitro immunotherapy model, patient derived organ-

oids, bispecific antibody, colorectal cancer.

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## A098 / The antitumor activity of a DNA vaccine encoding glioma-associated antigens is enhanced by surgical resection in a GL261 glioblastoma orthotopic model

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DNA vaccination against cancer has become a promising strategy for inducing a long-lasting and specific immune response. However, the activity of DNA vaccines is limited when used as single therapy. To enhance their activity, DNA vaccines can be combined with other therapies that can drive the vaccine-induced immune response into the tumor microenvironment. (1, 2)

This work evaluates the potential synergic effect of a DNA vaccine encoding glioma-associated antigens (GAAs) and tumor resection and their ability to reduce the onset of glioblastoma recurrences, in an orthotopic model. Hence, a DNA vaccine encoding the GAAs TRP2 and gp100, expressed by GL261 glioblastoma cells, has been designed and delivered by electroporation in the tibialis muscle of mice 16, 23 and 29 days after the GL261 orthotopic inoculation. A significant increase in the survival of vaccinated mice that underwent surgical resection compared to the controls (vaccine or resection) was observed. Immunological analysis by flow cytometry and ELISpot show a significant decrease of the infiltrated immunosuppressive cells 13 days after the priming dose of the vaccine (at day 29 post-tumor inoculation) and the presence of antigen-specific and immunologically active T cells in the brain when the two therapies are combined.

Hence, the combination between the glioblastoma tumor resection and DNA vaccine immunotherapy can increase mice survival in a clinically-relevant preclinical model, opening the door to a new standard of care for GBM patients.

Keywords: DNA vaccines, glioblastoma, resection, orthotopic model.

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## A099 / GEFH1 signalling upon microtubule destabilisation is required for dendritic cell activation and specific anti-tumor responses

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Dendritic cell (DC) activation is a critical step in the induction of anti-tumour T cell responses in cancer. DC maturation conventionally is triggered by the engagement of pattern recognition receptors (PRRs) [1]. More recently it has been described that anti-neoplastic chemotherapy that targets microtubule dynamics, such as ansamitocin P3, induce DC maturation. As a therapeutic consequence, the targeted delivery of microtubule destabilising agents (MDAs) can induce potent anti-cancer adaptive immunity, which can be further boosted by immune checkpoint inhibitors [2, 3]. However, the distinct immune activation in DCs operational downstream of microtubule destabilisation remain elusive. Here we demonstrate that chemotherapy capable of microtubule destabilisation has direct effects on DC function, namely induce potent DC maturation and elicit anti-tumour immunity. We utilised RNAseq to better characterise the intracellular signalling pathways and transcriptional responses upon microtubule destabilisation in DCs. RNAseq analyses revealed the extent and specificity of the guanine nucleotide exchange factor-H1 (GEFH1) dependent immune response in DCs in the context of microtubule destabilisation. In response to chemotherapy, GEFH1 drives a distinct cell signalling program dominated by the JNK pathway and the AP1/ATF transcriptional response for the control of innate and adaptive immune responses by DCs. Animals lacking GEFH1 signalling were unable to efficiently cross-present antigens to CD8 T cells upon microtubule destabilisation and consequently were more refractory to therapy-induced anti-tumour immunity. We also observed more rapid growth of untreated MC38 tumours in GEFH1 deficient animals compared to wildtype, indicating that GEFH1 is required for anti-tumour immune response. Our study identifies an alternate intracellular axis in DCs induced upon microtubule destabilisation in which GEFH1 promotes protective anti-tumour immunity. Dendritic cell activation by the GEFH1 pathway may be utilised to overcome the immune tolerant tumour environment and also improve the utility of current immune checkpoint blockade and personalised cancer vaccinations.

Keywords: GEFH1, Dendritic Cells, Microtubule-targeting agents, Cross presentation.

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## A100 / Vaccination against a neoepitope whose expression is driven by acquired resistance to oncolytic virotherapy prevents VSV-resistant tumour recurrence.

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We have shown that treatment of established tumours with oncolytic Vesicular Stomatitis Virus (VSV) leads to emergence of recurrences following initial tumour regression. Escape from virotherapy, is explained, in part at least, because VSV infection induces IFN-ß-dependent expression of the mutagenic cytidine deaminase APOBEC3B (Huff et al., Mol. Ther. Oncolytics, 2018. 11:1-13). Here, we show that whole genome sequencing of tumour cells which escaped VSV oncolysis showed multiple genomic mutations with an APOBEC3B mutational signature (TCA-TTA or TCT-TTT), about 300 of which were in coding regions. Of these, >90% of cells which escaped VSV-IFN-ß oncolysis in vivo expressed a mutated version of the CSDE1 gene, showing that APOBEC3-mediated mutation of CSDE1 exerts a strong selective pressure to facilitate escape from oncolysis. One mutation in the CSDE1 gene was predicted to mutate an H2Kb/ H2Db MHC binding epitope such that it would bind with a significantly higher affinity (<500nM) than the unmutated wild type epitope. Whilst C57Bl/6 mice were completely tolerant to the wild type CSDE1 epitope, a relatively strong T cell response was raised against the point mutated CSDE1* epitope in mice vaccinated with CSDE1*-pulsed DC (~200pg IFN-g /ml/106 splenocytes). Mice with B16 tumours treated with intra-tumoral VSV-IFN-ß underwent significant regressions, followed by aggressive recurrence. Mice treated additionally with CSDE1-pulsed DC following i.t VSV-IFN-ß (+/- anti-PD1) did not survive any longer than mice treated with VSV-IFN-ß alone (median survival of 42d vs 48d). However, mice treated with i.t. VSV-IFN-ß and then vaccinated with CSDE1* pulsed DC survived significantly longer (median survival 75d) and,

with anti-PD1, long terms cures were generated (no recurrences in over 70% of mice). Immune correlative studies confirmed that cured mice had generated potent T cell responses against CSDE1*. To date, we have not observed autoimmunity directed against normal cells expressing unmutated CSDE1 in these cured mice. Taken together, our data show that tumour recurrences can express specific mutations which drive escape from frontline therapy, a proportion of which may generate novel, immunogenic neo-epitopes. By combining sequencing data to identify heavily selected mutations in treatment-resistant recurrences, with predictive bioinformatic analysis for possible neo-epitope formation, it is possible to ambush recurrences which have been trapped/forced into a phenotype that facilitates escape from frontline therapy but is itself readily targeted by concomitant vaccination.

Keywords : Oncolytic Viruses, VSV, NEO Antigens, Tumour Recurrence.

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Huff et al., Mol. Ther. Oncolytics, 2018. 11:1-13

## A101 / PBRM1 loss reduces IFN gamma-STAT1 activity and promotes resistance to immunotherapy in renal cell carcinoma

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Polybromo-1 (Pbrm1), encoding a mammalian specific subunit of Switch/Sucrose Non fermenting (SWI/SNF) chromatin remodeling complex, is the second most frequently mutated gene in clear cell renal cell carcinoma (ccRCC). The impact of PBRM1 mutation on response to immunotherapy in patients with renal cell carcinoma (RCC) has become a topic of intense debate. Here we employed both RCC patient samples and an immune competent murine RCC model to assess the influence of tumor cell PBRM1 loss on IFN gamma-STAT1 signaling, the tumor microenvironment (TME) and response to immune checkpoint blockade. PBRM1/Pbrm1 deficiency impaired IFN gamma-induced phosphorylation of JAK2 and STAT1, and the subsequent expression of downstream target genes involved in TME modulation, such as Cxcl9, Icam1, Irf1, and Stat1 itself. Pbrm1 knockout in murine RCC Renca cells impaired the binding of BRG1, the ATP-dependent enzyme subunit of SWI/SNF complex, to the promoter of IFN gamma receptor 2 (Ifngr2) and reduced Ifngr2 expression. In both human and murine RCC tumors, PBRM1/Pbrm1 loss was associated with a less immunogenic tumor phenotype as characterized by reduced T cell infiltration, downregulated checkpoint activity and a decreased IFN gamma-related expression profile. Furthermore, Pbrm1 deficient Renca subcutaneous tumors in mice demonstrated longer latency but more resistance to PD-1 blockade, either with early treatment at day four after cell inoculation or with late treatment when tumors reached 100-200 mm3. This study provides key insights into the TME and clinical characteristics of ccRCC patients with PBRM1 mutations, and forms a framework for future mechanistic and clinical studies on the interaction between genomic features in RCC and response to immunotherapy.

Keywords : PBRM1, IFN gamma, Tumor microenvironment, Renal cell carcinoma.

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## A102 / Targeted delivery of CCL21 chemokine with nanocomplexes promotes tumor-infiltration of T lymphocytes

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In the past decade, breakthroughs in cancer immunotherapy have led to unprecedented clinical responses in patients. However, treatment of many solid malignancies with highly immunosuppressive local microenvironment remains challenging. Tumors develop a number of mechanisms to prevent T-cell infiltration, one of the key factors associated with good clinical outcomes, has been shown to be critical for many effective cancer immunotherapies. Therefore, a safe and effective strategy to improve T-cell infiltration is highly desired to enhance immunotherapies against solid tumors including adoptive T-cell transfer (ACT) therapy. Chemokines are known to be essential in orchestrating T-cell trafficking and tumor infiltration. Chemokine deprivation is one of major contributing factors for effector T-cell exclusion in tumors. Reprogramming chemokine profile in tumors could be a promising strategy for enhancing T-cell infiltration. However, applicable and efficient and delivery Methods for chemokines are still lacking. Chemokines are small-size proteins (8-15kDa) with highly positive surface; fast renal clearance and non-specific binding to glycosylaminoglycans (GAGs), negatively charged polysaccharides that are universally abundant in the extracellular matrix (ECM), contribute to the major hurdles in establishing a desired chemokine gradient in tumor. Here, we devised a reversible charge shielding strategy by complexing CC21/Fc, a Fc fusion protein of chemokine, with a negatively charged biocompatible polymer, such as heparin, a naturally occurring GAG, for tumor-targeted delivery of chemokines. We found heparin could efficiently shield the positive charge of CCL21/Fc forming a nanocomplex with zeta potential of -9.92 mV and a hydrodynamic size of 48.6 3 16.4 nm. The CCL21/Fc-heparin nanocomplexes were shown to accumulate in tumors efficiently compared to native CCL21/Fc by taking advantage of enhanced permeability and retention (EPR) effect while avoiding unspecific binding to ECM in circulation upon intravenous injection. The nanocomplexes disassembled specifically inside tumors likely due to the overexpression of heparan sulfate in tumor vasculature, which has higher chemokine binding affinity than heparin, leading to established chemokine gradient for T-cell chemotaxis along tumor vasculature and hence tumor-infiltration of T-cells. In a syngeneic B16F10 mouse melanoma model, we have shown that the CCL21/Fc-heparin nanocomplexes dramatically increased the tumor infiltrating T-cells and enhanced the efficacy of ACT therapy without overt toxicities. This is a potentially generalizable strategy for efficient delivery of various chemokines or chemokine combinations into solid tumor for reprogramming tumor microenvironment.

Keywords : Chemokine, Tumor infiltration, Adoptive T-cell therapy, Drug delivery.

## A103 / Mouse and human PD-L1 in the in vitro immune checkpoint blockade assay – functional similarities and differences in druggability

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The aim of this study was to introduce human and mouse cell

lines into the in vitro immune checkpoint blockade assay in order to provide a platform for the testing of PD-L1-blocking molecules. PD-L1 (B7-H1, CD274) is a natural ligand for the programmed cell death-1 (PD-1, CD279) protein. PD-1 is expressed mostly on the activated T cells, and together with PD-L1 constitute one of the most important immune checkpoints that are known to inhibit activated T cells. PD-L1 is overexpressed in many cancers and contributes to the cancer escape from the immune surveillance. For this reason, the PD-1/PD-L1 immune checkpoint became an important molecular target of anti-cancer therapies. Currently, four anti-PD-L1 and two anti-PD-1 monoclonal antibodies (mAb) are used in the treatment of cancer patients, in many cases bringing improvement of the patient's survival and limiting adverse side effects compared to classical therapies. The development of non-mAb inhibitors of PD-1/PD-L1 interaction may further improve ICB therapy. However, more relevant cell-based models are still required for the early, pre-clinical functional evaluation of the activity of such molecules. In our study, we introduce the modified PD-1/PD-L1 immune checkpoint blockade assay, where mouse and human cancer cell lines expressing elevated endogenous PD-L1, and bearing a stable expression of the T cell receptor activator (TCR-A) are contacted with either artificial Jurkat T cell effector cells or PBMCs to provide a measurable PD-1/PD-L1 immune checkpoint. Then, we test several classes of therapeutic and experimental PD-1/PD-L1 blockers, i.e. therapeutic antibodies, small molecules, macrocyclic peptides, and short peptides, in order to verify their ability to block human or mouse PD-L1. Our study shows that while mouse PD-L1 interacts with human PD-1 and forms a functional immune checkpoint, particular molecules designed to bind to human PD-L1 fail to block mouse PD-L1. Our model provides an easy method, that may help to decide whether the PD-L1-blocking compound could be tested in classical syngenic mouse models, or should it be directed for testing with the use of more advanced humanized models. The presented results may thus help in the development of new, non-mAb classes of molecules targeting PD-L1.

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Keywords: immune checkpoint blockade (ICB), PD-1/PD-L1, cell-based model.

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## A104 / Circulating suppressive immune cells predict the efficacy of anti PD-1 immunotherapy in patients with advanced non-small cell lung cancer

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The major suppressive immune cells in tumor sites are myeloid derived suppressor cells (MDSCs), tumor associated macrophages (TAMs), and regulatory T (Treg) cells, and the major roles of these suppressive immune cells include hindering T cell activities and supporting tumor progression and survival. In this study, we analyzed the pattern of various circulating suppressive immune cells and cytokines in patients with non-small cell lung cancer (NSCLC) to see whether those suppressive immune cells hinder T cell activities leading to poor clinical outcomes. Baseline blood samples were collected from stage I to IV NSCLC patients (n=59), and baseline and one week after the therapy paired blood samples were collected from stage IIIB to IV NSCLC patients (n=83) undergoing anti PD-1 immunotherapy either pembrolizumab or nivolumab. The efficacies of peripheral blood suppressive immune cells along with CD39+CD8+ T cells individually or collectively in anti-PD-1 immunotherapy were evaluated using flow cytometry and T cell suppressive assay. Granulocytic-MDSCs (G-MDSCs), monocytic-MDSCs (M-MDSCs), TAMs, Treg cells, and CD39+CD8+ T cells increased according to NSCLC stages, and MDSCs effectively suppressed T cell activities and induced T cell exhaustion ex vivo. Further, the analysis of 83 NSCLC patients treated with anti-PD-1 immunotherapy demonstrated that low G-MDSCs (PPFS = 0.03, Pos = 0.04), M-MDSCs (PPFS = 0.04, Pos = 0.005), TAMs (PPFS = 0.007, Pos = 0.01), and CD39+CD8+ T cells (PPFS = 0.57, Pos = 0.02) were associated with longer progression-free survival (PFS) and overall survival (OS) compared with high groups. When we performed combined analysis of three suppressive immune cells, G-MDSCs, M-MDSCs, and TAMs collectively, patients who had low frequency of all three suppressive immune cells showed more prominent difference of PFS (6.7 months vs 2 months; P = 0.006) and OS (8.5 months vs 4.2 months; P = 0.004) compared to patients with high levels of all three suppressive immune cells. The analysis of 83 advanced NSCLC patients treated with anti-PD-1 immunotherapy demonstrated that G-MDSCs, M-MDSCs, TAMs and CD39+CD8+ T cells frequencies in peripheral blood individually and collectively might be useful as potential predictive biomarkers.

Keywords: Myeloid-derived suppressor cell, Tumor associated macrophage, Non small cell lung cancer, Immune checkpoint inhibitor.

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## A105 / Poor outcome with anti-PD-L1 antibody due to undesirable pharmacokinetic properties in PD-1/PD-L1 blockade sensitive mouse models

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Objective: Recently anti-PD-1 antibodies (aPD-1 Abs) and anti-PD-L1 (aPD-L1) Abs have been approved for cancer treatment. Even though aPD-1 and aPD-L1 Abs target same PD-1/PD-L1 axis, it has not been verified that both Abs are able to exert equivalent pharmacological activity in patients whose tumors are sensitive to PD-1/PD-L1 blockade therapy. In this study, we analyzed the difference between both Abs in blood concentration, biodistribution and degradation by using aPD-1/PD-L1 Abs labeled with radioiso-

topes (In-111/I-125) in tumor-bearing mice which were sensitive to PD-1/PD-L1 blockage to evaluate relationship between pharmacokinetics (PK) and therapeutic effects.

Method: Tumor bearing mice were prepared by subcutaneous inoculation with mouse colon cancer MC38 cells or mouse breast cancer MM48 cells. In pharmacological studies, Abs were intraperitoneally (i.p.) injected into tumor-bearing mice at 50-200 ug/mouse at day 5, 8, and 12 after tumor-inoculation. Tumor volume was measured to evaluate tumor progression. Abs were labeled with In-111 via chelate agents, and labeled with I-125 through covalent bond. The labeled Abs were i.p. injected into tumor-bearing mice at low dose (2 ug) or high dose (200 ug). Tumors and organs were harvested at several time points after the injection up to 216 hrs, and radio activities in organs were measured by a gamma counter. The accumulation of Abs were expressed as % of injected dose/g organ. Because In-111 tends to be accumulated in organs due to poor permeability and I-125 was eliminated from organs rapidly due to high permeability, the ratio of I-125 and In-111 could reflect the degradation of Abs after cellular uptake.

Result and Discussion: aPD-1 Ab showed anti-tumor effect in both MC38 and MM48 tumor models, which showed that both models were sensitive to PD-1/PD-L1 blockade. However, aPD-L1 Abs showed lower anti-tumor effect in MC38 models, and negligible effect in MM48 tumor bearing mice at tested doses. It was observed that aPD-1 Ab circulated in the blood for long time and accumulated in tumors. On the other hand, aPD-L1 Ab was largely accumulated in normal tissues, especially in the spleen and liver, which resulted in low blood concentration and tumor distribution. Because PD-L1 expresses in not only tumors but also normal organs abundantly, large dose should be required to deliver aPD-L1 Ab to tumors by saturating antigen-antibody interaction in normal organs in the case of aPD-L1 Ab. As we expected, aPD-L1 Ab with high dose accumulated in tumors as much as aPD-1 Ab. However, aPD-L1 Ab was degraded faster than aPD-1 Ab, which resulted in poor inhibition of target and led the less anti-tumor effect.

Conclusion: According to PK studies, aPD-1 Ab represented linear PK, while aPD-L1 Ab showed non-liner PK between low and high doses. Collectively, the pharmacokinetics of aPD-1/PD-L1 Abs which target the same axis were not equivalent, which resulted in poor outcome with aPD-L1 Ab compared with aPD-1 Ab. The selectivity of expression of target molecules in both normal tissues and tumors should be considered to optimize their therapeutic efficacy.

Keywords: PD-1/PD-L1, pharmacokinetics, pharmacodynamics.

## A106 / DNA barcoding demonstrates immuno-editing of metastatic breast cancer cells at the clonal level

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The majority of cancer patients die of metastatic disease. Currently, immunotherapy is one of the few successful treatment modalities for metastatic disease and can lead to very durable responses in some cancer types such as melanoma. Unfortunately, many cancers including breast cancer exhibit resistance to the currently approved immunotherapies and patients invariably relapse (1). We hypothesize that innate resistance of cancer cells can explain some of this resistance.

To test whether clonal selection of innately resistant cells can drive resistance to immunotherapy, we used cellular DNA barcoding, a powerful technique that allows for the analysis of clonal dynamics over time. We introduced a DNA barcode library (ClonTracer) into a polyclonal murine metastatic breast cancer cell line (4T1). These barcodes are then “read” using next-generation sequencing. Following orthotopic transplantation of these barcoded cells, the primary tumor was resected and metastases allowed to develop. Comparison of wildtype mice with immunocompromised NSG mice demonstrated the immune system did not affect barcode number or distribution in the primary tumors. In contrast there was a significant increase in the number of barcodes in the metastatic lungs of NSG mice, despite the level of disease burden being identical at ethical endpoint. We then compared wild-type mice treated with either adjuvant immunotherapy (anti-PD1+anti-CTLA4) or with control antibodies, this revealed that the number of unique metastatic clones was significantly reduced following immunotherapy. Further analysis revealed a subset of highly metastatic clones detected in the lungs of NSG mice were reduced in abundance in wild-type mice and completely eliminated following immunotherapy, these exact clones were detected in replicate mice suggesting that this is a pre-existing phenotype. Conversely, we identified another subset of resistant clones that were highly enriched following immunotherapy. We are currently identifying what makes these clones more or less susceptible to immune surveillance. This study shows that there are subsets of breast cancer cells that are innately more resistant to immunotherapy. We now aim to identify tumor-intrinsic pathways regulating recognition by the immune system that can be targeted to improve immunotherapy response in breast cancer patients.

Keywords: Breast cancer, Metastasis, Immunotherapy.

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P. Schmid et al., Atezolizumab and Nab-Paclitaxel in Advanced Triple-Negative Breast Cancer. New England Journal of Medicine 379, 2108-2121 (2018).

## A107 / Targeting tumor-associated macrophages in osteosarcoma: depletion versus re-direction.

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The pro-tumorigenic role of tumor-associate macrophages (TAM) has been widely demonstrated in several tumor types. On the contrary, very few data exist on the activity of TAM and of other immune cells in osteosarcoma (OS), the most common primary bone tumors in young adolescents and children.

We have recently demonstrated in immuno-competent OS mouse models that trabectedin, a marine-derived chemotherapic agent, exerts a potent anti-tumor activity that is further enhanced by the combination with anti-PD-1 antibody. Besides directly affecting neoplastic cells, trabectedin modifies the tumor immune landscape by recruiting T lymphocytes at the tumor site. However, contrary to what expected from the literature, the treatment did not affect TAM.

To better investigate the role of TAM in OS, we performed a “proof-of-concept” co-injection experiment with OS cells mixed with macrophages differentiated in vitro toward classical M1 or M2 phenotype, or left undifferentiated (M0). While the presence of M1 macrophages inhibited OS, neither M0 nor M2 macrophages affected significantly tumor growth. This result indicated that the presence of TAM per se does not influence OS growth and suggested that their re-direction toward a M1 phenotype could exert therapeutic activity. To clarify this issue, mice bearing OS tumors on both flanks were treated locally, only in one lesion, with either liposome-encapsulated clodronate to deplete TAM, or with SD101, a synthetic oligonucleotide with immunostimulatory CpG motifs. Despite clodronate was efficient in reducing TAM infiltration, tumor growth inhibition was limited, on both flanks; on the other hand, SD101 efficiently halted the growth of both treated and untreated lesions. TAM number was not affected by SD101 treatment, but they showed a significant reduction in the expression of the M2 marker CD206. Additionally, tumor infiltration by CD8 T cells was enhanced in both treated and untreated tumors by SD101, but unaffected by clodronate.

Overall these preliminary results support the hypothesis that re-directing the phenotype of TAM in OS could be therapeutically more efficient than their direct elimination.

Keywords: osteosarcoma, macrophages, tumor microenvironment.

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Ratti C et al. Clin Cancer Res. 2017 Sep 1;23(17):5149-5161. doi: 10.1158/1078-0432.

## A108 / TGFb blocks IFNα/b release and tumor rejection in spontaneous mammary tumors

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Type I interferons (IFN) are being rediscovered as potent anti-tumoral agents. Activation of the STimulator of INterferon Genes (STING) by DMXAA can induce a strong production of IFN and the rejection of transplanted tumors. In the present study, we asked whether targeting STING with DMXAA leads to the regression of spontaneous MMTV-PyMT (Spont-PyMT) mammary tumors. We show that these tumors are refractory to DMXAA-induced regression. This is due to a blockade in the phosphorylation of IRF3 and the ensuing IFNa/b production. Our study identified TGFbeta abundant in spontaneous tumors, as a key molecule limiting DMXAA-induced tumor regression. Finally, blocking TGFb promoted infiltration of Spont-PyMT tumors by activated MHCII+ tumor-associated macrophages and restores their ability to produce IFNα that enable tumor regression induced by STING activation. On the basis of these findings, we propose that type I IFN-dependent cancer therapies could be greatly improved by combinations including the blockade of TGFb.

Keywords: STING, TGFb, Macrophages, Type I IFN.

## A109 / An in vitro preclinical approach for safety and efficacy testing has supported two new ImmTAC® molecules into the clinic

Florence Schlosser (Immunocore Ltd).

ImmTAC (Immune mobilising monoclonal TCRs Against Cancer) molecules are a new class of soluble bispecific fusion protein that combine a T cell receptor (TCR)-targeting system with an anti-CD3 effector function to potently and specifically activate T cell responses against cancer cells. ImmTAC molecules have the potential to overcome many of the limitations of other immuno-oncology agents. By using TCRs to target intracellularly processed peptides presented by human leukocyte antigen (HLA), ImmTAC molecules can access cancer-specific proteins that traditional antibody-based therapies cannot currently target. Moreover, as ImmTAC molecules redirect polyclonal T cells to the tumour, they overcome dependence on pre-existing immunity within the tumour micro-environment.

ImmTAC molecules are human specific on both the targeting and effector ends, therefore animal models are inappropriate for testing safety and efficacy. A fully in vitro preclinical package has been developed to test efficacy, safety and specificity of ImmTAC molecules. The efficacy of ImmTAC molecules is tested by T cell activation (IFNγ release) and redirected T cell killing of cancer cells presenting the antigen (Granzyme B release, Caspase 3/7 activation) in the presence of ImmTAC molecules. To predict potential off-tumour activity, ImmTAC molecules are tested against a wide panel of HLA relevant tissue specific cells from healthy donors. In addition, ImmTAC molecules are tested for their potential to elicit platelet activation and cytokine release in whole blood to assess the risk of cytokine release syndrome caused by broad immune cell activation. Alloreactivity of redirected T cells is assessed against a comprehensive panel of cells covering a broad range of HLA types. Molecular analysis further assesses the specificity of ImmTAC molecules, by determining the TCR-binding motif and potential cross-reactive peptides. The preclinical data were sufficient to enable both IMCnyeso (in collaboration with GSK) and IMC-C103C (in collaboration with Genentech) specific for epitopes from the cancer testis antigens NY-ESO-1 and MAGE-A4 respectively, to gain approval for entry into first in human Phase I clinical trials for the treatment of a broad spectrum of solid tumour indications (NCT03515551 – IMCnyeso, NCT03973333 – IMC-C103C).

Keywords: bispecific, TCR-based therapy, preclinical, in vitro.

## A110 / Identifying targets for the next generation of immunotherapies; using large-scale multiomics to better explore the tissue environment

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The Human-Leucocyte Antigen (HLA) complex presents peptides derived from intracellular and extracellular proteins on the cell surface for sampling by T cells, via their T cell receptors (TCRs). Amongst the repertoire of HLA-peptides are a number of attractive targets for immunotherapy, including peptides derived from cancer-associated, viral, overexpressed or mutated proteins. Immunocore's oncology platform focuses on the development of affinity-enhanced TCR-based molecules that target HLA-peptides to activate highly potent and specific T cell responses against cancer cells. Identifying and validating viable HLA-peptide targets forms a crucial element of the platform. Here, we highlight the challenges faced in seeking to decode the immunopeptidome within a tissue microenvironment, and demonstrate that leveraging large scale immunopeptidomic libraries and data-led experimental approaches can unravel the complex relationship between mRNA, protein, and HLA-peptide. Our immunopeptidomic workflow focuses on the immunopurification and structural characterisation of HLA-peptides from immortalised cell lines and fresh frozen tissue. HLA-peptides are immunopurified using a variety of anti-HLA antibodies. After acid elution, desalting and fractionation by reversed-phase liquid chromatography, HLA-peptides are analysed by MS using different instruments. The exact experimental design utilizes a data intelligent approach based on measuring the absolute quantity of material recovered from immunopurification. Mass spectra are searched against the human proteome and integrated in an in-house database together with HLA typing data and gene expression. By integrating omics data from over 1000 individual experiments in 200 immortalised cell lines, we have dramatically increased the depth of the HLA ligandome captured, with up to 100000 peptides identified from a sample. This has enabled us to achieve near total coverage of the protein-coding genome, with over 90% of the proteome captured for the restriction HLA-A*02:01. Using techniques that complement each other has proved particularly beneficial, with gains of up to 20% in identification when a sample is analysed across 2 different MS instruments. Our comprehensive HLA class I peptide atlas is used as a reference tool to develop bespoke experimental designs for interrogating tissue material. By employing this strategy, we routinely identify over 10000 HLA-peptides in most tissue specimens which, although fourfold lower in yield compared to immortalized cell lines, is significantly higher than has previously been reported. We find a marked variation in peptide recovery yield across different tissue types, with this variation linked to the amount of HLA complex, rather than the quantity of tissue used in the experiment. The level of HLA-peptide complexes recovered from tumour is generally higher than that of the normal adjacent tissue, suggesting no significant global downregulation of class I HLA within primary tumour material. As our tissue dataset has expanded we have been able to demonstrate good alignment with our cell line atlas. Taking as an example gp100, we showed that the pool of HLA-A*02 peptides recovered from tumour tissue correlates better with data obtained from the analysis of cell line models than that from prediction algorithms. Additionally, individual peptide stoichiometries within the same gene correlate well

between datasets and overall the peptide novelty rate in tissue is low, with the majority of novelty attributable to genes that are not represented in immortalised cell lines. These findings suggest that in vitro cell models are broadly representative of the in vivo HLA-class I environment and highlight the power of using cell lines to form a reference that enables maximal value to be derived from the more challenging tissue environment.

Keywords: Mass spectrometry, Immunopeptidomics, HLA-peptide, T cell receptor.

## A111 / Defining the immunomodulatory effects of IMM-101: A promising, novel co-therapy for cancer.

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IMM-101 is a whole cell preparation of heat-killed Mycobacterium obuense (NCTC13365) currently undergoing clinical evaluation for the treatment of advanced pancreatic adenocarcinoma. With a 5-year survival rate of less than 5%, novel treatments for pancreatic cancer are a major clinical need. In a phase II trial, treatment with IMM-101 in combination with gemcitabine (one of the current standard-of-care options for pancreatic cancer) increased median survival from 4.4 months to 7 months in patients with metastasis (Dalgleish et al., 2016). Pre-clinical studies in syngeneic murine models have also found significantly decreased tumour burden when IMM-101 is administered in combination with the checkpoint inhibitor anti-PD-1, compared to anti-PD-1 alone. Establishing the mechanism by which IMM-101 causes this effect is key for advancing the use of this bacterial immunomodulator as a cancer therapy.

We generated dendritic cells (DCs) in vitro from murine bone marrow (BMDCs), or from human peripheral blood mononuclear cells (moDCs). We found that exposure to IMM-101 significantly increased the activation status of both human moDCs and murine BMDCs, and enhanced BMDC ability to process and present antigen to both CD4+ and CD8+ T cells in vitro. Subcutaneous injection of IMM-101 activated BMDCs into IFNγ-eYFP reporter mice showed that IMM-101 primed DCs induced strong IFNγ production by a variety of different immune cell populations, including CD4+ T cells, CD8+ T cells, NK cells, NK-T cells and γδ T cells. This IFNγ induction occurred independently of IL-12p70 secretion by IMM-101 primed DCs. Additionally, direct intradermal injection of IMM-101 into the ear pinnae of mice resulted in a significant increase in the number of both innate and adaptive immune cells within the skin draining lymph node. Further, this approach induced clear local and systemic immune responses, as both draining lymph node cells and splenocytes isolated from mice that received intradermal IMM-101 secreted significant levels of IFNγ following culture ex vivo.

These data elevate understanding of the potential mechanisms by which IMM-101 promotes anti-tumour immune responses, through the activation of DCs and induction of anti-tumour cytokines locally and systemically. Ongoing work is focusing on interrogating the specific character of the T cell response to IMM-101, along with establishing the mechanism of its systemic effects.

Keywords: Mycobacteria, Immunomodulators, Dendritic cells, Interferon-γ.

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## A112 / PD-L1:CD80 Cis-heterodimer Triggers CD28 While Repressing both PD-1 and CTLA-4 Pathways

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Combined immunotherapy with anti-PD-1/PD-L1 and anti-CTLA-4 has resulted in superior clinical responses compared to single agent therapy. The underlying mechanisms for this synergy have yet to be elucidated and investigations have largely focused on cellular interactions. Herein, we report a molecular crosstalk in which the PD-1 ligand PD-L1 and the CTLA-4/CD28 ligand CD80 heterodimerize in cis detected by both cell-free reconstitution system and cell-based assay. This heterodimerization inhibits both PD-L1:PD-1 and CD80:CTLA-4 interactions, but preserves the ability of CD80 to activate the T cell costimulatory receptor CD28. Remarkably, PD-L1 expression on antigen presenting cells (APCs) protects CD80 from CTLA-4 mediated trans-endocytosis, and the therapeutic PD-L1 blockade antibody atezolizumab paradoxically downregulates CD80 on APCs, presumably reducing its co-stimulatory ability. Importantly, this effect can be negated by co-blockade of CTLA-4 with ipilimumab. Our study reveals an unexpected immune stimulatory role of cis-acting PD-L1 and a mechanism of anti-PD-L1/anti-CTLA-4 crosstalk, providing a therapeutic rationale for combination blockade of PD-L1 and CTLA-4.

Keywords: PD-L1, CD80, Cis-interaction.

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Zhao, Y., Lin, C.-H., Lee, C., Xu, X., Huang, Z., Xiao, C., Bui, J., Lu, L.-F., and Hui, E. (2019). PD-L1:CD80 Heterodimer Triggers CD28 While Repressing Both PD-1 and CTLA4 Pathways. bioRxiv, 615138.

## A113 / EOS884448, a high affinity fully human antagonistic TIGIT antibody, with multiple mechanisms of action to boost anti-tumor immunity

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T cell Immunoreceptor with Ig and ITIM domains (TIGIT) is a T cell co-inhibitory receptor that has emerged as a key checkpoint driving tumor cell immunosuppression. Its expression is predominantly reported on Tregs, CD8+ T cells and NK cells from healthy individuals. In cancer patients, TIGIT expression is upregulated, and associated with exhaustion phenotypes, being frequently correlated with PD-1 expression. TIGIT cognate receptors are members of the poliovirus receptors, among which CD155 has the highest affinity for TIGIT, that are expressed on antigen presenting cells but also on different tumor types which provides a strong rationale for blocking TIGIT as a therapeutic approach to reverse T or NK cell dysfunction linked with cancer progression. The anti-tumor effects mediated by its antagonistic activity can be further enhanced by engagement of its fragment crystalizable (Fc) region with activating Fc gamma Recptors (FcgR) that contribute to deplete TIGIT+ Tregs, described as highly immunosuppressive.

EOS884448 was selected among a panel of antagonist anti-TIGIT antibodies, using a yeast display library of fully human antibodies and characterized for their sub-nM affinity to primary T cells and anti-tumor properties. Its high binding affinity to cynomolgus TIGIT allows for its direct use in GLP Tox studies in non-human primates.

EOS884448 antagonistic activity potently reverts TIGIT inhibition and restores pro-inflammatory cytokine release in presence of CD155 ligand. This mode of action was studied in an IL-2 driven reporter-based system as well as in primary CD8 T cells from healthy individuals and patients suffering from different solid tumor indications.

The analysis of its potential to trigger antibody-dependent

cell-mediated cytotoxicit (ADCC) was evaluated in primary samples from either healthy donors or cancer patients by comParison of different isotype formats. EOS884448, as an hIgG1, shows preferential depletion of Tregs over memory CD4+ and CD8+ T cells.

Similar observations were made in syngeneic tumor mouse models. The treatment with an ADCC enabled anti-mouse TIGIT surrogate on tumor bearing mice leads to significant anti-tumor responses in monotherapy and in several combinations. Analysis of dissociated mouse tumors showed increased frequency of activated tumor infiltrating T effector cells, while the frequency of tumor infiltrating Tregs was decreased.

To support selection of cancer indications for EOS884448, TIGIT and CD155 expression were characterized in both circulating and tumor-infiltrating lymphocytes as well as in tumor cells, by flow cytometry and immunohistochemistry (IHC).

Analysis performed by flow cytometry, on matched samples of circulating and tumor-infiltrating immune populations from 12 cancer patients highlighted the overexpression of TIGIT associated with cancer. Tumor-infiltrating Tregs represented the population with the highest TIGIT expression, findings further confirmed by IHC.

These data strongly supports the use of an isotype with high affinity for activatory FcgR in cancer patients who could benefit from a drug acting simultaneously through depletion of highly suppressive Tregs and restoration of T and NK cell effector functions within tumor.

Keywords: TIGIT, Immuno-oncology, Regulatory T cells.

References:
Anti-TIGIT antibodies: Patent Pub. No.: WO/2019/023504

## A114 / Localized radioimmunotherapy via intratumoral nanofluidic drug-eluting seed for enhanced immunotherapy efficacy

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Considering the modest response to systemic immunotherapy along with toxicity issues, we posit that sustained intratumoral delivery could improve therapeutic index by locally modulating tumor immune microenvironment for a robust antitumor immune response. However there has yet to be an effective approach for sustained local drug delivery to avoid repeated intratumoral injections. Here we present an intratumorally implantable device termed nanofluidic drug-eluting seed (NDES) to deliver immunotherapy directly in the tumor in a sustained manner. NDES-mediated drug delivery occurs via passive diffusion through nanochannels, which controls drug release without the need for actuation, analogous to an hourglass. Given the poor efficacy of monotherapies, we investigated the synergistic efficacy of sustained intratumoral CD40 immunotherapy antibody via NDES in combination with radiotherapy using the 4T1 mouse model of triple negative breast cancer. Tumors were irradiated with 8 Gray for 3 consecutive days followed by intratumoral implantation of NDES with CD40 antibody. We fluorescently-labelled CD40 antibody to track drug localization in the tumor via fluorescence IVIS live animal imaging, comparing NDES-mediated sustained intratumoral delivery to systemic intraperitoneal and direct bolus intratumoral injection. We demonstrated using IVIS imaging, flow cytometry and histological analysis that sustained intratumoral immunotherapy via the NDES confined immunotherapy to the tumor and modulated the tumor immune microenvironment with minimal systemic drug exposure. Overall, we present the NDES as an effective localized drug delivery platform for improving immunotherapy efficacy with minimal toxicity.

Keywords: drug delivery, immunotherapy, breast cancer, radiation.

## A115 / Targeting mechanisms of protein degradation to improve VSV immunotherapy

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Viral immunotherapy aims to induce robust anti-cancer immune responses by combining direct delivery of a tumor antigen from a viral vector with strong innate inflammatory activation. While these therapies have shown safety and efficacy in a clinical setting, a large proportion of non-responding patients indicates the need for continued development of these modalities. We have previously reported that the systemic delivery of vesicular stomatitis virus (VSV) expressing truncated forms of the tumor associated antigens NRAS, Cytochrome-C1, TYRP1, or hgp100 provide superior therapeutic efficacy in treating B16 melanoma tumors compared to VSV expressing the full-length forms of the proteins. Mapping the truncated regions, roughly 20 amino acids at the C-terminal ends, onto structural domains of each protein revealed that the C-terminal truncation removes the cytosolic and/or transmembrane domains thus disrupting the stability and localization of each protein. Indeed, in vitro analysis of TYRP1 protein stability indicated that the truncated form of the antigen has an altered localization within the cell, by immunofluorescence, and a reduced half-life of less than 4 hours, as measured by radiolabeled pulse-chase. From these data, we hypothesized that modulation of protein stability and degradation may improve immunogenicity of tumor antigens. We therefore investigated how modulation of global protein degradation pathways or targeted antigen destabilization affects antigen presentation and immunogenicity. First, we characterized how modulation of broad cellular degradation pathways such as the unfolded protein response pathway (UPR) influences antigen degradation, epitope presentation and immunogenicity. We found that treatment of murine B16 cells expressing chicken ovalbumin protein (OVA) with the small molecule UPR activator Thapsigargin (Tg), induced degradation of the OVA antigen, and enhanced the presentation of the immunogenic epitope SIINFEKL. This correlated with an improved recognition by cognate OTI T cells in vitro leading to enhanced activation, proliferation, and cytokine expression measured by flow cytometry and intracellular cytokine staining. Next, we generated destabilized OVA protein mutants through structure-directed amino acid mutation to understand the relationship between protein stability, degradation, and immunogenicity. We identified one OVA mutation which promoted degradation and resulted in significantly enhanced presentation of the SIINFEKL epitope on H2Kb molecules. This enhanced antigen presentation resulted in better recognition, activation, and proliferation of OT-I T cells in vitro. Ongoing studies seek to identify the therapeutic benefits of expressing degradation-prone antigens from our VSV vectors. This research has significant impact on our understanding of how modulation of protein stability and degradation affects the efficacy of our immunotherapeutic platforms.

Keywords: Antigen presentation, Immunotherapy, VSV.

## A116 / A novel tri-valent T cell engager targeting tumor endothelial marker 1 (TEM1, Endosialin) efficiently recruits human T cells to kill endogenous tumor cells

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Recent advances in immunotherapy have revealed the potential of human T cells to control tumor growth. One clinically validated paradigm for harnessing the cytotoxic potential of T cells involves the use of bi-specific antibody-derived molecules comprising a tumor-targeting moiety together with an associated 'bridging' anti-CD3 recognition domain. This strategy has been applied successfully to treat hematological malignancies, but T cell re-targeting to solid tumors remains challenging, partly due to insufficient tissue penetration. This obstacle could be bypassed by selectively targeting the supporting tumor neo-vasculature. Tumor endothelial marker 1 (TEM1, Endosialin) is specifically upregulated in the stroma and vasculature of many solid tumors, while being largely undetectable in healthy tissues.

In order to target TEM1, we have performed a phage display discovery campaign and successfully isolated a panel of fully human scFv binders from a naïve human antibody library. Extensive screening, biophysical characterization, affinity measurements and functional binding assays identified a set of specific scFv candidates recognizing different domains of TEM1. Clones with desirable properties were subsequently fused to an anti-CD3 recognition domain in order to engineer T cell engaging bispecific mediators. In this context, we have incorporated both anti-CD3 and anti-TEM1 domains into a stable, tri-valent bridging molecule that permits either bi-valent or bi-paratopic recognition of TEM1 on the surface of target cells. Using our diverse panel of anti-TEM1 scFvs, we have been able to vary the affinity and avidity of these engagers in order to achieve an optimal selectivity threshold for endogenous TEM1 targeting. Further, we have also explored the utility of targeting TEM1 epitopes both distal and more proximal to the membrane, aiming to identify the impact of epitope location on the efficiency of T cell engagement.

Our results show that these novel engager molecules specifically redirect human T cells to TEM1 expressing target cells. Stimulation with our bi-specifics activates primary human T cells selectively in the presence of TEM1 positive cells, leading to the secretion of effector cytokines and lysis of tumor cells. Dissecting the contribution of scFv affinity, targeting avidity and epitope distance helped us identify the ideal molecular properties to bridge T cells and their TEM1 expressing targets. Moreover, in vivo studies aiming to investigate the potential of the most promising T cell engager molecules to suppress tumor growth are ongoing.

In summary, we have developed a novel tri-valent T cell engager targeting TEM1 with the aim to explore the therapeutic potential of redirecting T cells to selectively kill TEM1 expressing tumor neo-vasculature.

Keywords: T cell engaging bispecifics, bispecific antibody, tumor endothelial marker 1, neovasculature.

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## A117 / Blockade of β-adrenergic receptor signaling improves cancer vaccine efficacy through its effect on naive CD8+ T-cell priming

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β-adrenergic receptor (β-AR) signaling has been shown to exert pro-tumoral effects by acting directly on tumor cells and angiogenesis. Growing evidence also suggests that β-AR expressed by immune cells affect the associated anti-tumor immune response. However, how and where β-AR signaling impinges the anti-tumor immune response is still unclear. Using a mouse model of vaccine-based immunotherapy, we show here that propranolol, a non-selective β-blocker, strongly improves the efficacy of the vaccine by enhancing the frequency of CD8+ T lymphocytes infiltrating the tumor (TILs). However, propranolol has no obvious effect on the reactivity of CD8+ TILs, a result further strengthened by ex-vivo experiments showing that these cells are insensitive to AR signaling triggered by adrenaline or noradrenaline. In contrast, we show that naive CD8+ T-cell activation is strongly inhibited by β-AR signaling and that the beneficial effect of propranolol mainly occurs during their initial priming phase in the tumor-draining lymph node. We also demonstrate that the differential sensitivity of CD8+ TILs and naive CD8+ T cells to β-AR signaling is linked to a strong down-regulation of β2-AR expression related to their activation status, since in vitro-activated CD8+ T cells behave similarly to CD8+ TILs. These results reveal that the initial priming phase of the anti-tumor CD8+ T-cell response is a decisive part of the suppressive effect of β-AR signaling on the immune response against cancer. These findings provide a rationale for the strategic use of clinically available β-blockers in patients to improve cancer immunotherapies such as anti-cancer vaccination strategies.

Keywords: Neuroimmunology, Vaccine-based immunotherapy, Adrenergic receptor, CD8+ T cells..

## A118 / Elucidating the mechanisms of action of anti-CD20 monoclonal antibodies using intravital imaging

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Rituximab, a monoclonal anti-CD20 antibody has revolutionized the treatment of B cells malignancies when it first came out in 1997. It targets the extracellular CD20 protein largely expressed by normal and malignant B cells and was shown to induce tumor depletion in vitro through various Fc-dependent mechanisms such as complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) or phagocytosis (ADP) (1, 2). Surprisingly, there is little evidence about its mode of action in vivo. Despite its clinical success, some patients relapse or are unfortunately resistant to treatment. Thus, there has been an aspiration to develop therapies endowed with improved efficacy. However, understanding where and how different anti-CD20 Abs may potentiate therapeutic responses in vivo is a critical step for generating new therapeutic antibodies. We and others (3, 4) have identified the liver as a site of ADP for circulating B cells following mouse anti-CD20 administration. Here, using mouse liver transplant, we reveal that the liver is critical for mediating efficient systemic B cells depletion following anti-CD20 treatment. Relying on intravital two-photon imaging of human CD20-expressing mice, we provide evidence that ADP by Kupffer cells (KC) is a major mechanism for rituximab-mediated B cell depletion. Notably, a glycoengineered anti-mouse CD20 Ab but not its wild-type counterpart triggered potent KC-mediated B cell depletion at low doses. Distinct thresholds for KC phagocytosis were also observed for GA101 (obinutuzumab), a humanized glycoengineered type II anti-CD20 Ab and rituximab(5). Finally, as tumor cells poorly recirculate and instead stably reside in tissues we next addressed whether anti-CD20 can deplete non-circulating tumor cells. Using a novel in vivo phagocytosis reporter,

we report here visualizing phagocytosis of lymphoma cells in the bone marrow of tumor-bearing mice following anti-CD20 therapy. Thus, we conclude that enhanced phagocytosis of circulating B cells by KC represents an important in vivo mechanism underlying the improved activity of glycoengineered anti-CD20 mAbs and that anti-CD20-mediated depletion of tumor cells also occurs at the tumor site albeit less efficiently. Understanding how clearance of non-circulating tumors could be enhanced during anti-CD20 therapy would be certainly beneficial for future therapeutic strategies.

Keywords: anti-CD20 monoclonal antibody, B cell malignancies, intravital imaging, phagocytosis.

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## A119 / 4224, a tumor exosome-transforming antibody targeting CD40 and EpCAM, induces superior anti-tumor effects compared to monospecific CD40 antibody

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4224 is a human CD40 x EpCAM bispecific, Fc silenced IgG1 antibody generated in the novel RUBY™ format. 4224 was developed with the purpose to generate an antibody capable of transforming extracellular vesicles (EV), such as exosomes, into autologous neoantigen delivery vehicles that are efficiently taken up by dendritic cells (DC). 4224, a tumor exosome-transforming antibody, has the potential to induce superior cross-presentation of tumor neoantigens present in EV and generate a broad tumor-specific T cell repertoire with the capacity to efficiently eradicate tumor cells. The mode-of-action of 4224 is dependent on a high expression of EpCAM on tumor-derived EV, as well as many solid tumors, and the ability of CD40 to mediate a superior cross-presentation of tumor antigens, compared to other DC targets. Dual targeting of EpCAM and CD40 would thus mediate an increased uptake of tumor EV by DC and provide a CD40-mediated activation of DC resulting in efficient cross-presentation of the tumor neoantigens within EV to CD8+ T cells.

The function of 4224 was first evaluated in vitro using human monocyte-derived DC, co-cultured with cells expressing EpCAM. DC activation was analyzed by flow cytometry measuring HLA-DR and CD86 expression on the DC and by measuring IL-12p40 levels in the supernatant. The results demonstrated that 4224 mediates EpCAM cross-linking-dependent activation of DC. The ability of 4224 to mediate internalization of tumor-derived EV by antigen-presenting cells (APC) was investigated using a live imaging system, measuring internalization of EpCAM+ tumor debris by CD40-expressing APC. In this setting, 4224 improved internalization of tumor debris by the CD40-expressing APC. In vivo, the anti-tumor efficacy of 4224 was determined in human CD40 transgenic mice bearing MB49 bladder carcinoma tumors transfected with human EpCAM. 4224 displayed a potent, EpCAM-dependent anti-tumor effect with significantly reduced tumor growth and improved survival compared to an equivalent dose of a CD40 monospecific antibody. The tumor-localizing property of 4224 also shows potential for improved safety compared to CD40 monospecific antibodies.

In conclusion, 4224 is a tumor exosome-transforming antibody targeting CD40 and EpCAM, which has the potential to mediate an expansion of the tumor-specific T cell repertoire, resulting in increased T cell infiltration and potent anti-tumor effects in patients with tumors expressing high levels of EpCAM.

Keywords: CD40, EpCAM, bispecific, antibody.

## A120 / Detection of neoantigen-specific CD8+ T-cells in patients with metastatic urothelial carcinoma treated with α-PD-L1 antibody

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Patients with metastatic stage IV urothelial carcinoma have previously had scarce treatment options and poor prognosis when treated with traditional chemotherapy, with overall survival averaging 9-15 months and only 5 % of patients surviving past 5 years. Recently, checkpoint inhibition immunotherapy involving α-Programmed Death-Ligand 1 (PD-L1) antibodies has shown great success in treatment of metastatic urothelial carcinoma patients. However, not all patients benefit from treatment, thus presenting a need for predictive parameters for patient treatment and clinical outcome when selecting patients to undergo α-PD-L1 treatment. Here, we investigate how neoepitope T-cell recognition contributes to clinical reactivity, and work to determine how neoantigen-specific T-cell population characteristics can be applied as a potential biomarker and predictive parameter for preselection of which patients will benefit from α-PD-L1 treatment.

In this study, we are investigating CD8+ T-cells recognition of mutation-derived neoantigens by screening blood samples from patients before, during and after treatment with α-PD-L1 antibodies. The patient cohort includes 24 patients with stage IV urothelial carcinoma, all of which have also previously been treated with chemotherapy. The patients have had varying clinical outcomes of the α-PD-L1 treatment. Based on whole exome sequencing of tumor and germline DNA, personal libraries of between 200 and 587 potential neoantigens have been predicted for all patients. Out of a combined 56 patient HLA types, potential neoantigens have been predicted towards 31 HLA types, averaging four HLA types per patient. The patient samples are screened with DNA-barcode-labelled pMHC-multimers, which allows high-throughput screening of all potential patient-specific neoepitopes in one parallel reaction.

Data from the first 12 patients screened shows that neoantigen specific T-cells could be detected in all patients. T-cell recognition of between one and 23 neoepitopes was observed across patient samples, with large variation in the number of detected neoepitopes between patients. Across all screened patients, detected neoepitope responses ranged from one to 15 at the beginning of treatment. During treatment of patients not responding to therapy, between zero and 16 detected responses were observed per time point. Non-responding patients did not seem to experience an increase in detected neoepitope responses over the course of treatment. For all non-responders, fewer detected neoepitope responses were seen at the terminating time point, ranging from zero to nine detected responses, compared to before treatment initiation. Concurrently, treatment-responding patients experienced either an increase in detected responses during treatment, to a maximum of between three and 23 responses per time point, or a persistence of up to several detected responses throughout the course of treatment, up to 166 weeks after treatment commencement. The dynamics of detected neoepitope responses thus seem to vary between non-responding patients and patients responding to treatment.

The findings from this study will provide insight into the dynamics of T-cell recognition of mutation-derived neoantigens in urothe-

lial carcinoma over the course of α-PD-L1 treatment, and may improve the understanding of how T-cells recognize, control and eliminate metastatic urothelial carcinoma during therapy. Furthermore, the successful characterization, and potential differentiation, of detected neoepitope responses between responders and -non-responders to therapy may facilitate a more accurate predictive parameter for clinical outcome prediction during patient treatment option preselection.

Keywords: Urothelial carcinoma, anti-PD-L1, Neoantigen T-cell recognition.

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## A122 / Intra-mammary tumor application: A superior model system to subcutaneous tumor cell inoculation

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Since decades syngeneic and xenograft tumor models are used in cancer drug development. In most cases tumor cells are applied subcutaneously because of the ease of tumor cell inoculation and tumor growth monitoring. Yet, the location of the tumor inoculation site is not precisely defined differing from person to person leading to potential differences in growth characteristics and tumor ulceration occurs frequently especially in syngeneic models. How can the subcutaneous injection route be improved? Is there a functional organ that allows a simple tumor cell application with an easy monitoring by calipering?

We extensively tested tumor cell inoculation into the mammary fat pad and compared this heterotopic application to the subcutaneous injection route. In all tested syngeneic models, tumor ulceration was prevented by almost 100% when injecting the tumor cells into the mammary fat pad. Ethical abortion criterion was primarily tumor volume. In contrast, animals of the subcutaneous tumors were mainly euthanized due to tumor ulceration. In addition, intra-mammary tumor growth showed less heterogeneity compared to the subcutaneous application. Both findings support the 3R rules with a refinement by strongly reducing tumor ulceration and a reduction in animal consumption due to more homogenous growth.

Beside these superior tumor growth characteristics of all investigated intra-mammary models, the CT26wt tumor model was intensively characterized by flow cytometry using an all-in-one flow cytometry staining panel to investigate all major immune-cell populations. Here the number of isolated cells per gram tumor mass was more than doubled in the intra-mammary tumors suggesting a higher stroma content. This is supported by the flow cytometry data which revealed that during the first 10 days of tumor development approximately half of the cells are leukocytes, whereas in the subcutaneous tumors the leukocyte population is little at the beginning increasing to only one quarter after 10 days.

Finally, the immune checkpoint inhibitor treatment was tested and found comparable between the intra-mammary and subcutaneous model. LL-2 tumors did not respond to anti-mPD-1 and anti-mCTLA-4 treatment in both application routes, subcutaneous and intra-mammary, whereas CT26wt and MC38-CEA tumor growth was reduced by anti-mCTLA-4 treatment for both cell application routes. Yet, the inhibitory effect of anti-mCTLA-4 treatment was overall less pronounced in the intra-mammary MC38-CEA and CT26wt model with no complete remission for the CT26wt model.

In conclusion, the heterotopic intra-mammary implantation of tumor cells was found to be superior to the traditional subcutaneous implantation resulting in improved meaning- and powerful tumor models.

Keywords: syngeneic tumor model, tumor ulceration, subcutaneous, mammary fat pad.

## A123 / Antitumoral control of MHC class I deficient tumors by combination therapy

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During response to immunotherapy, T cells elicit selective pressure, which may give rise to tumor cell escape clones that are no longer recognized and eliminated by the immune system. In this regard, defective antigen presentation through loss of MHC class I has been repeatedly observed as a mechanism of acquired resistance, especially in highly immunogenic tumors such as melanoma. (1) Our aim is to understand how MHC class I deficient tumors behave differently in vivo compared to MHC class I proficient tumors and to establish translationally relevant approaches to tackle MHC class I loss as a mechanism of acquired resistance.

To this end, we generated MHC class I deficient tumor models by genetic knock-out of b2m in mouse tumor cell lines considered to form immunogenic or non-immunogenic tumors in vivo. We then performed a comparative characterization of MHC class I proficient versus deficient tumors and found that MHC class I deficiency results in a growth benefit for originally immunogenic tumors, which bear an inflamed tumor microenvironment (TME) that becomes immunologically cold in the absence of MHC class I. The lack of antigen presentation to CD8 T cells significantly diminishes the amount of IFN-γ expressed in the TME. This leads to lower induction of interferon-inducible chemokines, consequently impeding the attraction of several potentially antitumoral immune cell subsets. In contrast to that, subsets regarded as immunosuppressive are mostly unaffected. This observation and the fact that many cancer therapies at least partially rely on immunity prompted us to undertake a comprehensive analysis of MHC class I loss as a mechanism of resistance against different therapies. We confirmed that MHC class I deficiency completely abrogates the antitumoral effect of T-cell based immunotherapies including immune checkpoint blockade. In addition to that, the loss of MHC class I also prevents a therapeutic response to chemotherapy or radiotherapy. Finally, we were able to establish a combination immunotherapy that facilitates therapeutic immune responses against MHC class I deficient tumors, resulting in complete tumor rejection and long-term survival of the majority of treated mice.

Keywords: MHC class I, Acquired Resistance, Immune Escape.

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## A124 / Arenavirus-based Vector Platform For Massive Tumor Self-antigen-specific CD8 T Cell Immunity

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The induction of powerful CD8+ T cell immunity to tumor associated self-antigens (TAAs) represents a critical yet challenging goal. Here we report on the development of an arenavirus-based delivery platform meeting this challenge. Previously we have shown that genetically engineered replication-attenuated lymphocytic choriomeningitis virus (LCMV) vectors, TheraT(LCMV), induce strong TAA-specific CD8 T cell immunity, but these responses can not be substantially augmented upon TheraT(LCMV) readministration. Counter to expectations, vector-neutralizing antibodies were not accountable for limited homologous prime-boosting capacity. Instead, dominant viral backbone-reactive CD8+ T cells competed against subdominant TAA-specific responses, limiting their magnitude. Herein we engineered and characterized delivery systems based on the arenaviruses Mopeia, Candid#1 and Pichinde (TheraT(MOP), TheraT(CAND), TheraT(PICV)) and demonstrate that heterologous TheraT(CAND) – TheraT(LCMV) and TheraT(PICV)-TheraT(LCMV) prime-boost substantially augment TAA-specific CD8 T cell responses by rendering them immunodominant. Accordingly, intravenous administration of mice triggered up to 50% TAA epitope-specific CD8+ T cells and cured established tumors. Conversely, TheraT(MOP) – TheraT(LCMV) prime-boost was poorly immunogenic owing to cross-reactive T cell epitopes in the respective viral backbones. These findings establish heterologous arenavirus prime-boost combinations as a powerful new modality in tumor immunotherapy and highlight CD8 T cell epitope dominance as a significant hurdle to overcome in the vectored delivery of TAAs.

Keywords: Arenavirus, Viral vector, HOOKIPA.

## A125 / Natural Killer Cells Correlated with T cell Biomarkers for Response to Immunotherapy in Advanced Hepatocellular Carcinoma

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Introduction: Checkpoint inhibitors targeting tumor-infiltrating T cells to trigger anti-tumor immune response have enamoured as the promising therapy. Majority of immunotherapy biomarker studies are focusing on T cell. However, investigation of other immune cell clusters may also yield crucial information for immunological changes. Guidance for precise immunotherapy as well as applicable response markers are currently limited. Henceforth, we are interested in whether Natural Killer (NK) Cells may also have the potential to serve as predictors for immunotherapy in advanced Hepatocellular carcinoma (HCC).

Method: Patients demographic along with clinical annotations are shown in Table 1. Healthy volunteers and newly diagnosed patients who were not exposed to chemotherapy or immunotherapy at the time of sample collection.

Results: One-way Analysis of Variance (ANOVA) in using PD-1, Tim-3 and OX40 as response predictor showed an effect on both T Cells (Fig1 A, C, D) and NK Cells (Fig2 A, C, D). No significant differences were observed in CTLA-4 (Figure 1B, 2B respectively) expression among cohorts on both T Cells and NK Cells. To understand the relationship between biomarkers on NK Cells and T Cells, pearson product-moment correlation coefficient was conducted between PD-1, Tim-3 and OX40. Tim-3 on T Cells showed a significant positive correlation to the same expression count on NK Cells (R= 0.9747, p <0.001), while OX40 also showed a significant positive correlation to the expression count on NK Cells (R= 0.9892, p <0.001). No significance in either PD-1 expression or CTLA-4 expression were seen in T Cells and NK Cells.

Keywords: Immunotherapy, T cells, NK Cells, Biomarker.

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## A126 / Blocking tumor-associated immune suppression with BAY-218, a novel, selective aryl hydrocarbon receptor (AhR) inhibitor

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Tumor cells co-opt multiple pathways in order to evade attack by infiltrating immune cells. One such mechanism is the upregulation of indole-2, 3-dioxygenase (IDO1) and/or tryptophan-2, 3-dioxygenase (TDO2), both of which are first-step, rate-limiting enzymes degrading tryptophan to the immunosuppressive metabolites kynurenine (KYN) and kynurenic acid (KA). KYN and KA bind and activate the aryl hydrocarbon receptor (AhR), which is expressed in many cell types and is well known for its immunosuppressive effects. Targeting of the AhR with an inhibitor may therefore provide a novel immunotherapeutic approach for enhancing anti-tumoral immune responses and treating cancer.

Here we describe the identification and functional immune characterization of BAY-218, a novel, selective and potent AhR small molecule inhibitor. Mechanistically, BAY-218 inhibited AhR nuclear translocation, dioxin response element (DRE)-luciferase reporter expression and AhR-regulated target gene expression induced by both exogenous and endogenous AhR ligands.

In vitro, BAY-218 rescued TNFa production from KA-suppressed LPS-treated primary human monocytes. Furthermore, BAY-218 enhanced T cell cytokine production in a human mixed lymphocyte reaction (MLR) and a mouse antigen-specific bone-marrow-derived dendritic cell (BMDC)-OT-I T cell co-culture. In the MLR, BAY-218 increased anti-PD1 antibody-mediated IL-2 and IFNg secretion, while an IDO inhibitor did not, indicating that BAY-218 is able to block AhR activation mediated by ligands outside of the IDO-KYN pathway. In vivo, BAY-218 enhanced anti-tumoral immune responses and reduced tumor growth in the syngeneic mouse tumor models CT26 and B16-OVA. FACS analysis of leukocytes infiltrating B16-OVA tumors demonstrated that administration of BAY-218 increased the frequency of tumor-infiltrating CD8+ T cells and NK cells while decreasing GR1+ myeloid cells and CD206+ M2 macrophages. Furthermore, BAY-218 enhanced therapeutic efficacy of an anti-PD-L1 antibody in the CT26 model.

In summary, AhR inhibition with BAY-218 stimulates pro-inflam-

matory monocyte and T cell responses in vitro and drives anti-tumor immune responses, resulting in decreased tumor growth, in vivo. Thus, inhibiting AhR represents a novel immunotherapeutic approach for blocking AhR-mediated tumor-associated immunosuppression.

Keywords: Aryl hydrocarbon receptor (AhR), Immunotherapy, Oncometabolite, Small molecule inhibitor.

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## A127 / TheraT-E7E6, a live-attenuated LCMV-based vector for active immunotherapy of HPV16+ cancer

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Active immunization against cancer requires the induction of exquisitely potent tumor-specific CD8+ T cell (CTL) responses, which can be repeatedly boosted and reactivated. Hookipa Pharma engineered a replication-attenuated viral vector platform (TheraT) based on the arenavirus lymphocytic choriomeningitis virus (LCMV), which meets these aforementioned criteria. Here, we present a preclinical data package of Hookipa´s lead immunotherapy product HB-201, targeting HPV16-driven cancer.

TheraT-E7E6 encodes a highly immunogenic, non-oncogenic version of the human papilloma virus 16 (HPV16) oncoproteins E7 and E6. Attenuation and safety of TheraT-E7E6 were demonstrated by i) rapid viral clearance after systemic administration of the vector and ii) reduced neurovirulence in mice.TheraT-E7E6 can be administered intravenously as systemic therapy or intratumorally as local therapy. Treatment was shown to induce substantial CD8+ T cell expansion and high frequencies of E7- and E6-specific CTL responses with a balanced effector / central memory profile. These responses were boosted upon TheraT-E7E6 re-administration. TheraT-E7E6 eradicated palpable tumors in a syngeneic mouse TC-1 tumor model of HPV-driven cancer. Even in mice with large tumors (~300mm3) TheraT-E7E6 afforded significant tumor control and improved survival, with high frequencies of E7-specific CTLs persisting for several weeks. Animals which cleared the tumor after TheraT therapy were long term protected from tumor re-challenge. Furthermore, TheraT has been shown to synergize in combination with checkpoint inhibitors (CPIs).

In conclusion, replication-attenuated TheraT-E7E6 is safe, highly immunogenic and shows excellent therapeutic efficacy as mono- or combination therapy in a preclinical model of HPV-induced cancer. After a successful IND filing, clinical trials for the treatment of HPV16-driven cancers will be initiated in 2019.

Keywords: Active Immunotherapy, HPV16+ cancer, CD8+ T cell (CTL) responses, Replication-attenuated viral vector platform.

## A128 / Comparing the immune environment of primary non-small lung tumors and brain metastases: implications for immunotherapy

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In non-small cell lung cancer (NCSLC), brain metastases (BM) are commonly found at time of diagnosis, and up to half of all NCSLC patients eventually develop BM during their clinical course. Although BM are associated with worse prognosis and altered quality of life, their tumor microenvironment is not well understood, and patients with BM are excluded from immunotherapy trials. Limited studies reveal significantly lower tumor-infiltrated lymphocytes (TILs) and PD-L1 expression in BM than in primary lung tumors. As immunotherapy with anti-PD-1/PD-L1 has become the standard of care in NSCLC, we explore whether the immune microenvironment of BM differs from synchronic lung primary tumors and whether biomarkers associated with response in BM can be identified.

85 BM and 14 matched lung primary tumor pre-treatment biopsies were taken prior to treatment from patients with treatment-naïve NSCLC who were seen at Neurosurgery Unit of Hospices Civils de Lyon from 2013 to 2018. Of these 85 patients, 24 were treated with nivolumab (n = 17), pembrolizumab (n = 5), or a combination of nivolumab and ipilimumab (n = 1), and the ORR of the cohort was 25% (RECIST 1.1). RNA was extracted and profiled with the NanoString PanCancer IO360 panel (NanoString Technologies, Inc). 45 gene signatures describing the immune response, tumor biology, and the tumor microenvironment, including the Tumor Inflammation Signature (TIS), an investigational 18-gene signature of a suppressed adaptive immune response that enriches for clinical response to pembrolizumab were calculated for each sample,

We compared the immune environment of all primary lung tumors and all BM in a combined analysis. Unsupervised hierarchical clustering revealed the presence of 3 distinct phenotypes based on immune signature scores, and BM were enriched in the cold cluster (92% in cold cluster vs 58% in hot cluster). Using differential expression analysis, BM were observed to have lower signature scores for multiple immune cell types vs lung tumors, including B cells (-1.7 log2 FC, $p < 0.001$), mast cells (-2.7 log2 FC, $p < 0.001$), T cells (-1.3 log2 FC, $p < 0.001$), and numerous other cell types. Additionally, we observed significantly lower scores for signatures associated with cytotoxicity, IFN-γ, IDO1, CTLA-4, PD-L2, TIGIT and PD-1 expression. No specific genes were upregulated in BM. In paired analysis, CCL21 chemokine was the most downregulated genes in BM (-4.7 log2 fold change, $p < 0.001$).

We then examined the association of the signatures with response to immunotherapy. Despite the lower immune infiltrate within the BM when compared to lung tumors, higher immune signatures scores in the BM were associated with response. In an unpaired analysis, multiple immune signatures scores were higher in the BM of responders when compared to the BM of non-responders, including TIS (1.46 log2 FC, $p < 0.001$), IDO1 (1.81 log2 FC, $p < 0.001$), and PDL1 (1.77 log2 FC, $p < 0.001$). TIS was also associated with response to anti-PD1 in BM by ROC curve analysis (AUC=0.824). RAD50 (-0.76 log2 FC, $p < 0.001$) and P4HA2 (-1.08 log2 FC, $p = 0.006$) were the most downregulated genes in responders compared to non-responders. Characterization of the immune landscape of BM of NSCLC is important to provide mechanistic insight into response to therapy as well as to identify new therapeutic targets. By profiling a cohort of BM and matched primary tumors, we identify location-specific transcriptional differences between the tumor sites. Furthermore, we demonstrate that the same immune signatures are associated with response to immunotherapy in BM as in primary tumors, despite an overall reduced immune presence in the BM. CCL21, involved in leukocyte cell trafficking, may represent a novel therapeutic target for BM. Future validation studies will provide support for these preliminary observations.

Keywords: Immunotherapy, Brain metastasis, Non small cell lung cancer, Microenvironment.

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## A129 / AL008 enhances myeloid anti-tumor function by inhibiting SIRPα signaling and activating Fc receptors.

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The SIRPα-CD47 axis represents a myeloid checkpoint in cancer. Tumor cells upregulate expression of CD47 to suppress innate immune cell effector responses through SIRPα signaling on tumor-infiltrating myeloid cells. Consistent with this view, high expression levels of SIRPα or CD47 correlate with worse overall survival in several cancers. Recent clinical data with CD47-targeted therapies that competitively block the interaction with SIRPα show clinical responses in some patients with hematologic and solid cancers when combined with anti-tumor antigen antibodies. However, known limitations associated with targeting CD47, such as antibody-mediated clearance of red blood cells (RBCs) and disruption of the SIRPγ-CD47 interaction important for T cell activation, likely hinder the anti-tumor immune response necessary for robust clinical benefit. Due to the restricted expression of SIRPα, a therapeutic advantage may be realized by targeting SIRPα and promoting immunostimulatory pathways that drive anti-tumor immunity. Here, we describe the discovery and characterization of a SIRPα-specific antibody, AL008, a first-in-class inhibitor of SIRPα that simultaneously antagonizes SIRPα signaling and stimulates Fcγ receptor (FcγR) activation. AL008 is a non-competitive inhibitor that triggers SIRPα internalization from the cell surface and subsequent degradation. Unlike other competitive antagonist antibodies to SIRPα, which require combination with opsonizing antibodies to promote tumor cell phagocytosis, AL008 stimulates tumor cell engulfment by macrophages in the absence of opsonizing antibody due to intrinsic FcγR activation. Moreover, the epitope of AL008 is conserved in all allelic variants of SIRPα but is not present in most common variants of SIRPβ and SIRPγ. Because of its restricted binding specificity to SIRPα, AL008 promotes dendritic cell-mediated stimulation of T cell proliferation by preserving CD47-SIRPγ axis, which contrasts with anti-CD47 antibodies and other anti-SIRPα/γ cross-reactive antibodies. Despite FcγR engagement by AL008, in vitro ADCC assays and in vivo studies in non-human primates verify that AL008 does not deplete myeloid cells or RBCs, which suggests a significantly improved safety profile in patients. Lastly, inhibition of tumor growth by AL008 in mouse models coincides with downregulation of SIRPα and induction of activation markers on tumor-associated macrophages. Thus, AL008 potentiates the anti-tumor effector functions of macrophages and dendritic cells in vitro, as well as in vivo models. This dual mechanism of AL008 provides a novel therapeutic strategy for targeting myeloid cells for immune activation.

Keywords: SIRPα, CD47, Fc receptors, macrophages.

## A130 / Pan-cancer analysis of allele-specific HLA-I loss suggests widespread occurrence across a diverse range of tumor types

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Neoantigen presentation arises as a result of tumor-specific mutations and is a critical component of immune surveillance. Decreased ability to present neoantigens due to gene-level copy number loss of the human leukocyte antigen (HLA) class I genes HLA-A, -B, or -C (HLA-I) can promote immune evasion. Notably, HLA-I loss has recently been described as a frequent phenomenon in non-small cell lung cancer but widespread analyses across varying tumor types is lacking.

To assess the frequency of HLA-I loss pan-cancer, we performed comprehensive genomic profiling on over 90 different tumor types comprising 100, 999 unique patient samples, mostly from tumors of patients with advanced/metastatic disease. HLA-I genotyping was conducted by OptiType and previously described copy number modeling techniques were applied to determine allele-specific loss of HLA-I genes by either homozygous deletion or loss of heterozygosity (LOH).

Loss of at least one HLA-I allele was detected in 30% of samples. Homozygous deletion of both HLA-I alleles was uncommon and seen in only 5% of all samples. The prevalence of HLA-I loss varied across tumor types (10-56%), with the highest prevalence in thymic carcinomas (56%), pancreatic islet cell tumors (53%), and squamous cell carcinomas (SCC) of various anatomic sites of origin (32-54%). The lowest prevalence was seen in melanoma (15% of cutaneous and 13% of intraocular), small cell lung cancer (14%), and Merkel cell carcinoma (10%). Mechanistically, HLA-I loss is expected to prevent the presentation of one or more neoantigens. Tumor subsets associated with high viral or neoantigen burden exhibited higher HLA-I loss, including human papillomavirus-infected head and neck SCC ($p<0.0001$, OR=1.7, Fisher's Exact) and microsatellite instable colorectal cancer ($p=0.002$, OR=1.4, Fisher's Exact). We also analyzed driver neoantigens and HLA-I loss. Overall, 14% (1585/11010) of samples with a KRAS G12 alteration were predicted to generate a KRAS G12 neoantigen (NetMHCpan). Of the samples with a predicted KRAS G12 neoantigen, 32% (502/1585) exhibited HLA-I loss. We found that neoantigens produced by KRAS G12 alterations preferentially bind to the lost allele in 87% (435/502) of HLA-I loss events ($p<0.0001$, Binomial Test) suggesting a strong selection bias against presentation of this neoantigen.

Overall, we assessed the landscape of HLA-I loss in over 100, 000 unique patient samples from a real-world genomics dataset. Prevalence of HLA-I loss varied greatly by tumor type with no clear association with mutational burden at the tumor type level. However, within a tumor type, highly mutated subtypes tended to exhibit increased HLA-I loss. This work also suggests HLA-I loss could be an effective mechanism of immune evasion when a driver mutation generates a neoantigen since loss of the driver would decrease tumor fitness. These results highlight the complexity of tumor-immune interactions and future studies need to be conducted to analyze the clinical impact of HLA-I loss on immunotherapy response rates.

Keywords: HLA, LOH, Neoantigens.

## A132 / Meta-analysis of the NanoString Clinical Transcriptomic Atlas Group (CTAG) Database to identify mechanisms of response to anti-PD1 treatment in melanoma and non-small cell lung cancer (NSCLC)

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Despite the approval of anti-PD1 blocking antibodies in a growing number of indications, the response rates in most indications remain disappointingly low. The availability of pretreatment biopsy samples from patients receiving immunotherapy for approved indications creates the opportunity to investigate biomarkers associated with response from real world cohorts. However, the small size of many such cohorts from single institute studies limits the power to generalize these results into larger patient populations. To address this challenge, we created a consortium of investigators who share clinical and accompanying transcriptional data

collected on the NanoString nCounter platform from patients treated with immunotherapy to foster scientific discovery and accelerate research. For the first time, we apply a meta-analysis to this combined cohort to identify correlates of anti-PD1 response in the transcriptional signatures from the tumor immune microenvironment to identify patients who are likely to experience clinical benefit.

Pretreatment tumor biopsies were collected from nine cohorts with metastatic melanoma (n = 52) or NSCLC (n = 114) initiating anti-PD1 therapy. RNA was profiled with the NanoString® IO360 and IO360 beta gene expression panels. 38 signatures that describe facets of the immune response, tumor biology, and the tumor microenvironment were calculated. Included in these signatures is the Tumor Inflammation Signature (TIS), an investigational 18 gene signature of a suppressed adaptive immune response which enriches for clinical response to pembrolizumab. Patients were analyzed by cancer type and clinical response, which was defined using both a binary categorization of response (RECIST 1.1 criteria) and overall survival. Variable clustering and sparse principal components analysis were applied to identify subsets of signatures that best explain the gene expression variation in the tumor samples. Penalized logistic regression and Cox proportional hazards Methods were used to model clinical response as a function of key signatures or principal components derived from the signatures.

Three major signature clusters were identified in both the melanoma and NSCLC subsets, representing inflammation-related signatures (lymphoid cells, IFNγ signaling, etc.), suppressive signaling (stroma, myeloid inflammation) and tumor metabolic activity (proliferation, hypoxia). In the final Cox proportional hazards model for the melanoma subset, both of the first two principal components (corresponding to inflammation-related signatures and tumor metabolic activity) showed significant association with overall survival (p = 0.012, PC1 HR: 0.88, 95% CI: 0.79-0.98; PC2 HR: 1.35, 95% CI: 1.02-1.78). This compared favorably to using TIS alone, which was not significantly associated with survival in this subset (p = 0.21, HR: 0.78, 95% CI: 0.53-1.15). For the NSCLC subset, logistic regression modeling based on categorical response suggested that TIS and SLC2A1 expression (from the hypoxia gene signature) could be combined to improve prediction of response, and association of SLC2A1 with survival was confirmed by analysis of lung cancer data in The Cancer Genome Atlas (TCGA). These findings suggest that, in both tumor types, combining immune signaling with information about tumor metabolism may improve our ability to predict response in anti-PD1 therapy .

In conclusion, this multi-cohort study allows for the development of multi-variate predictors of response to anti-PD1 monotherapy using a single research assay to transcriptionally profile the tumors. Using this model, we can generate robust predictions from real-world cohorts across multiple cancer indications, which may lead to the development of improved diagnostic assays to guide treatment decisions.

Keywords: Biomarkers, Meta-analysis, Anti-PD1.

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## A133 / The power of in vitro assays in immuno-oncology drug development

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During the last years, significant advancement has been made in the clinical application of cancer immunotherapies. Molecules directed against immune checkpoints and other agonists show great promise for treatment of a variety of malignancies. Next to the lymphocyte checkpoint modulators, a wide range of therapeutics with the potential to reverse the tumor-induced suppression are under development.

This increasing interest in the tumour microenvironment leads to focus on new bioassays to represent all the players of the cancer immune response. Early functionality screening of antibody-based checkpoint modulators can be done by in vitro assays using primary immune cells. Mixed lymphocyte reaction (MLR) assays using both innate cells and lymphoid cells mimic a real physiological T cell response and are widely used for the potency screening of candidate therapeutics. The use of different allogenic donor combinations can provide additional information on the profile of the responding population. Other T cell assays such as antigen-specific Cytomegalovirus (CMV) recall activation assays or Staphylococcal enterotoxin B (SEB) stimulation assays can be used to evaluate the ability of test molecules to promote T cell responses.

Other important players in the tumor microenvironment are the macrophages which possess important active and regulatory functions in both innate and adaptive immune responses. Classical activated macrophages, also classified as M1-like macrophages, comprise immune effector cells with an acute inflammatory phenotype while the alternatively activated M2-like macrophages have suppressive and healing capacities. Tumor associated macrophages (TAMs) are present at high densities in solid tumors and share many characteristics with so called M2 macrophages. Although distinguished classification and in vitro generation and polarization of M1- and M2-like macrophages is challenging, in vitro assays can be a first step to screen the effect of the test molecules on the phenotype and function of the macrophages. For example, macrophage precursors display extraordinary plasticity in response to exogenous and endogenous stimuli which can lead them to M2-polarized macrophages or towards the M1-activated status. Using in vitro polarization and functional macrophage assays, one can screen molecules with the potential to influence M1 and M2 like macrophage generation and polarization. Next to that, the effect of test molecules on the macrophage function can be evaluated using a macrophage suppressive assay. Here the ability of molecules to reverse the stimulating effect of M1-macropahges or the M2- macrophage suppressive effect can be determined.

Natural Killer (NK) cells also play a role in the anti-tumor immune response. NK cells kill tumor cells through different mechanisms, such as cytokines production or antibody dependent cell cytotoxicity (ADCC), which can be monitored in vitro.

Regulatory T cells (Treg) can also be found in the tumour microenvironment and present a highly suppressive phenotype. Their role in relation to cancer development and progression has shown to be important. Therefore, the ability of molecules to reverse the Treg suppressive effect on immune effector cells can be evaluated in vitro using these cell-type specific suppressive bioassays.

The use of bioassays contributes to a better understanding of the tumour microenvironment and the steps needed to generate an anti-tumour response by the immune system will help to assess the functional potential of new drugs, design clinical trials and ultimately discover relevant biomarkers.

Keywords: In vitro assys, T cell responses, Macrophage polarization, Regulatory T cells.

## A134 / ACM Nano vesicles as efficient delivery vehicles for cancer antigens to antigen presenting cells and improve survival in tumor models

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Our Artificial Cell Membrane (ACM) platform comprises inert block co-polymers that form nano vesicles with enhanced flexibility to incorporate whole proteins, individual peptides or nucleic acids. The platform has been successfully demonstrated as infectious disease vaccines where encapsulated antigens (both bacterial and viral) have significantly enhanced immune protection and thus survival in comParison to antigens alone and both with or without adjuvants. When administered with adjuvants, synergistic enhancement of the response was found for a range of adjuvants. No significant toxicities have been observed in both small (mice) and large (pigs) animal studies thus far. During the course

of developing animal vaccines, we hypothesized that the ACM platform improves the immunogenicity and efficacy by direct delivery of antigens to dendritic cells, leading to an enhanced T cell response. Hence, we sought to translate the use of the ACM platform for cancer vaccines by firstly, studying in detail the uptake of empty ACM vesicles in both murine and human peripheral blood mononuclear cells; and secondly, encapsulating cancer antigens in ACM vesicles for subcutaneous delivery of cancer vaccines in established cancer antigen models. We found that both murine and human myeloid derived antigen presenting cells (in particular certain subsets of dendritic cells) selectively take up fluorescently labeled empty ACM nano vesicles. In vivo models of prophylactic and therapeutic B16-OVA, B16F10, and MC-38 were used to test the efficacy of subcutaneous injections of ACM encapsulated cancer antigens in comParison to antigens alone or in combination with adjuvants. In prophylactic B16-OVA mouse models, ACM encapsulated OVA prevented the development of tumors, and remnant tumor cells showed significantly more tumor infiltration of OVA-specific T cells than for mice treated with OVA alone. In all the tested murine cancer models, ACM encapsulated antigens consistently increased antigen-specific T cells and anti-cancer response while improving survival significantly compared with antigens alone. Based on this data, we conclude that the ACM polymer nano vesicle platform improves delivery, and efficacy of cancer antigens in established murine models thus providing rationale to develop neoantigen cancer vaccines for humans with our ACM platform.

Keywords: Synthetic nano carriers, Delivery vehicle, Tumor Antigens, Neo Antigen.

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## A135 / Landscape of antigen-specific T cell responses in immune checkpoint inhibitor therapies in metastatic melanoma patients

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Cancer immunotherapies have shown great promise in the treatment of metastatic melanoma, but not all patients are responsive. Variations in the antigen-specifities of T cell clonotypes and their phenotypes could impact the treatment efficacy, but this is incompletely understood. To address this, we have built MelanoMAP database, which brings together three types of T cell repertoire data. We have gathered TCRβ-seq data from 399 longitudinal samples from 205 melanoma patients treated with five different immune checkpoint inhibitor modalities. We have also profiled and collected epitope-specific TCRβ-seq data and finally, scRNA+TCRαβ-seq data to understand unselected repertoire data better.

To understand the antigen recognition of T cells, we have analysed TCRβ sequences from pMHC-multimer sorted T cells against over 80 melanoma and viral epitopes. As TCR:pMHC -recognition is degenerate by nature, we have used an unsupervised learning strategy GLIPH 2.0 [1] that groups TCRs recognising the same antigens based on global and local amino acid similarities. Interestingly, T cell responses against two MART1-antigen epitopes, AAGIGILTV and ELAGIGILTV, showed striking similarity on their antigen binding, as these repertoires shared most of the antigen-contacting motifs. This finding was further validated with crystallography data, as a single TCR can bind both of these epitopes similarly with high affinity. For MELOE1 we could not find such convergence, elucidating that not all melanoma-antigens elicit homogenous responses.

After analyzing the antigen-specific signals from this data, we sought to define a machine-learning classifier that could be used as an in silico multimer-sorting strategy for our unselected data. For this purpose, we have leveraged TCRGP [2], our recently described Gaussian process method that can predict if TCRs recognize certain epitopes. We used the same multimer-sorted TCRβ sequences as training data and were able to build classifiers for the two MART1-peptides, AAGIGILTV (AUC=0.879) and ELAGIGILTV (AUC=0.739), but not for MELOE1 (AUC=0.617).

With our TCRGP classifiers, we were able to predict antigen-specific T cells from recently published scRNA+TCRαβ seq data from 25 melanoma patients[3]. 8.62% of the tumor infiltrating T cells were predicted to be reactive against any of the 5 shared melanoma epitopes and 5.19% against viral epitopes at FPR of 5%. The anti-melanoma clonotypes were enriched to dysfunctional CD8+ phenotype (p=0.019). Within the exhausted cells, the anti-melanoma T cells were more exhausted than anti-viral and expressed higher amount of immune checkpoint molecules (PDCD1, CTLA4 and LAG3) and had upregulated IFNα and IFNγ response pathways (p.adj=0.010 and p.adj=0.023). The anti-viral clonotypes expressed higher amount of memory markers (CCR7, GZMK) and were the most naïve-like of the exhausted cells which could enforce the role of the passer-by hypothesis of the anti-viral clonotypes in tumor environment.

Finally, we sought to identify antigen-specific T cells in our vast cohort of TCRβ sequenced patients. The abundance of clonotypes recognising shared melanoma antigens did not correlate with the amount of neoantigens. Surprisingly, in responders and non-responders no difference was observed in the expansion of anti-melanoma clonotypes. In contrast, in anti-PD1 non-responders, anti-viral TIL cells expanded more during the therapy than in responders (anti-influenza p.val=0.018), showing “off-target” effect of checkpoint-therapies.

In summary, with our in-depth TCR analysis of T cells in melanoma we were able to predict antigen-specific T cells, show their distinct transcriptomic states and discover similarities in the antigen binding of melanoma epitopes. Our results give novel insights into antigen-specific responses and how different immune checkpoint therapies modulate them.

Keywords: T cell, checkpoint blockers, single-cell RNA-sequencing, bioinformatics.

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## A136 / Impact of panel design and cut-off on tumor mutational burden (TMB) assessment in metastatic solid tumors

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Tumor mutational burden (TMB) has emerged as a promising biomarker to predict response to immune checkpoint inhibitors (ICIs) in a number of advanced solid cancers. TMB is defined as the number of mutations (somatic single (SNV) and multinucleotide variants (MNV) and small insertions and deletions (indels)) per megabase pair (Mb) of sequence examined and can be measured by genome, exome or gene panel sequencing. Since WES or WGS techniques are not routinely used in clinical practice, gene pan-

el-based targeted sequencing platforms have become feasible alternatives ready to be implemented in routine diagnostics. But harmonization of TMB reporting by various gene panel platforms is currently lacking. Moreover, effectiveness of ICI treatment is primarily described in metastatic disease. Since mutational patterns may differ between primary tumor and metastases, selection of patients for ICI treatment is ideally based on biomarker detection in samples from metastatic tumors. Therefore, the objective of our analysis was to assess the concordance between different gene panels in TMB measurements on metastatic samples.

The variety of TMB measured by 7 different gene panels was assessed based on whole genome sequencing as a reference. Data of 2 841 whole genome sequenced metastatic cancer biopsies was used to perform an in silico analysis of TMB determined by seven gene panels (FD1CDx, MSK-IMPACT, Caris, Tempus, Thermofisher, NeoGenomics and Kew) compared to exome-based TMB as a golden standard. For TMB determination, the number of variants (SNVs, MNVs and indels) within the panel design were divided by the panel footprint (0.78-1.48 Mb) or the size of the exome (30 Mb). Cut points for high TMB were simulated from 5/Mb to 40/Mb. The misclassification rate was derived from the sum of the percentages of false positive and false negative TMB measurements by each gene panel compared to exome based TMB. Secondly, the data was dichotomized with a cut point of 10 mutations/Mb to define high TMB. An receiver operating characteristics (ROC) analysis was performed to determine the threshold that each gene panel should set in order to classify most patients in the right TMB category.

The misclassification rate declines from up to 30% to less than 1% when the cut point is increased from 5 mutations/Mb to 40 mutations/Mb. Furthermore, misclassification rates are inversely correlated to the panel size. The largest panel size of 1, 48 Mb, containing 592 genes, corresponds to the lowest misclassification rates. ROC analysis demonstrated that for correct classification at 10/Mb the cut point for each panel design may vary more than 20% (7.8 to 11.7). More specifically, these cut points appear to be tumor type specific.

Here we show that, because of design differences, it is crucial to adjust the cut point for each gene panel platform. Therefore, a major limitation for the use of targeted sequencing platforms is the inter-assay variation and the need for dynamic thresholds to compare TMB outcomes for different platforms and cancer types. Specifically, trials that use TMB determined by a specific panel as a prospective selection biomarker for ICI response can only result in platform and disease specific patient selection. In conclusion, we would like to underscore the importance of whole exome- or whole genome-based mutation measurements of metastatic tumor samples for benchmarking TMB-based diagnostic biomarker platforms.

Keywords: tumor mutational burden, sequencing, gene panel, biomarker.

## A137 / Impact of tobacco smoking on immune microenvironment and outcomes in head and neck squamous cell carcinoma

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Tobacco contains many carcinogenic chemicals that contribute to the development of multiple cancers, including head and neck squamous cell carcinoma (HNSCC) [1]. Recently, immune checkpoint inhibitors (ICI) have been used to treat recurrent and metastatic HNSCC. However, only 10-20% of patients respond to ICI. In order to understand if smoking affects the tumor immune microenvironment and patients' response to ICI in HNSCC, we analyzed RNA and DNA sequencing data from The Cancer Genome Atlas (TCGA) and MSK IMPACT database [2]. We found that the mutational signature of tobacco exposure is associated with a strongly immunosuppressive tumor microenvironment in HNSCC. Additionally, the response rates to ICI are lower in smokers than non-smokers. FAT1 is the second most frequently mutated gene in HNSCC and has been identified as a tumor suppressor [3]. It has been proposed that FAT1 mutation impacts outcomes stronger in smokers than non-smokers [4]. To further analyze this hypothesis, we studied the subgroup of HNSCC patients carrying FAT1 mutations stratified by smoking status and found overall survival in smokers was significantly lower compared to non-smokers when treated with ICI. Taken together, our findings indicate that smoking induced mutational signature leads to immunosuppressive tumor microenvironment in HNSCC and negatively affects the patients' response to ICI and survival.

Keywords: head and neck squamous cell carcinoma, immune checkpoint inhibitor, tobacco smoking, tumor immune microenvironment.

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## A138 / An unbiased screen identifies a CD137xPD-L1 bispecific IgG1 antibody with unique T cell activation and binding properties

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CD137 (4-1BB) is a transmembrane costimulatory receptor on T and NK cells that enhances adaptive immune responses and is a critical mediator of antitumor immunity. CD137 signaling requires receptor clustering normally facilitated by the trimeric CD137 ligand (CD137L). Alternatively, CD137 signaling can be triggered either directly by agonistic monoclonal antibodies (mAbs) or indirectly via crosslinking of CD137 binding mAbs by $Fc\gamma$ receptors on neighboring cells. The development of CD137 targeted agents for cancer therapy has been hampered by on-target off-tumor toxicity in the case of agonist, monospecific, bivalent mAbs or limited antitumor activity in the case of crosslinking mAbs. To address the issues of toxicity and efficacy a highly selective and potent CD137xPD-L1 bispecific antibody (bAb) was identified by applying an unbiased functional screening approach. Collections of common light chain Fabs recognizing CD137 and PD-L1 were produced based on antibody panels from immunized MeMo® mice. A large and diverse panel of CD137xPD-L1 bAbs was then produced by combining different CD137 and PD-L1 Fabs based on epitope and sequence diversity in the IgG1 Biclonics® format. The bAbs were screened for activity in reporter cell lines expressing the receptors. This unbiased combinatorial screening identified a CD137xPD-L1 bAb (MCLA-145) for which CD137 mediated activation is dependent on the presence of PD-L1 on a neighboring cell and, as such, the antibody acts in 'trans'. Flow cytometry experiments demonstrated that MCLA-145 is fully cross-reactive to cynomolgus monkey CD137 and PD-L1. The CD137 Fab arm blocks the interaction of CD137 with CD137L as demonstrated in a competition assay by flow cytometry. The PD-L1 Fab arm blocks the interaction between PD-1 and PD-L1 as demonstrated in ELISA. Binding epitopes were mapped by shotgun mutagenesis using a flow-based screen. In addition, hydrogen-deuterium exchange experiments were performed to map the binding domain on CD137. Data show that MCLA-145 binds the ligand binding domain of CD137 domain (CRDII). Monovalent binding affinities were measured by surface plasmon resonance (SPR) and radioactive iodine labeling and demonstrated affinities in the low nM (CD137) and subnanomolar (PD-L1) range. SPR experiments also confirmed that MCLA-145 was able to bind simultaneously to both CD137 and PD-L1 recombinant proteins. The unique binding properties of MCLA-145 may result in an increased therapeutic window by specifically activating CD137 expressing cells in the tumor niche where PD-L1 is expressed while simultaneously blocking inhibitory input from the PD-1/PD-L1 axis.

Keywords: CD137, PD-L1, 4-1BB, Bispecific Antibodies.

## A140 / CTX471, a novel agonistic antibody targeting CD137, can eradicate very large tumors in vivo by selectively reprogramming the tumor microenvironment

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While antibody blockade of inhibitory checkpoint molecules has provided significant benefit to many cancer patients, most individuals will eventually fail checkpoint therapy and have their tumors progress. There is an intense effort underway to increase the number of patients that respond to cancer immunotherapy by discovering novel molecules that block inhibitory or agonize stimulatory receptors. CD137 (4-1BB) is a member of the tumor necrosis factor receptor superfamily (TNFRSF) that provides co-stimulatory signals to activated lymphocytes and monocytes. In preclinical models, CD137 agonism has demonstrated the ability to reduce tumor burdens and can be combined with existing and other novel therapies to increase therapeutic efficacy. However, clinical trial data with two CD137 agonist antibodies has been mixed; one antibody showed low efficacy with no dose-limiting toxicities, and the other displayed dose-limiting liver toxicities at sub-therapeutic doses. We have identified a novel agonistic IgG4 antibody against CD137, CTX-471, that induces potent anti-tumor activity without hepatic toxicity. CTX-471 recognizes a unique, non-ligand competitive epitope on CD137 that is shared by human, cynomolgus monkey, and mouse. In vitro, CTX-471 displayed an intermediate level of activity between the two clinical-stage anti-CD137 antibodies, had activity that was dependent on the presence of Fc-gamma receptors (FcR), and can synergize with CD137L engagement. In mice, CTX-471 demonstrates curative monotherapy activity in multiple syngeneic tumor models, including CT26, A20, EMT-6, and MC38OVA, and confers long-term protection from subsequent tumor rechallenge in 100% of cured mice. In therapeutic studies with very large (>500mm3) CT26 tumors, CTX-471 had the unique ability to cure mice where validated antibodies against PD-1, PD-L1, CTLA-4, OX40, and a different anti-CD137 clone had little to no effect. Additional in vivo and ex vivo studies with CTX-471 showed that this was due to a reprogramming of the tumor microenvironment and the ability to drive a unique mRNA program in T cells as compared to the super-agonist anti-mouse CD137 antibody clone 3H3. Unlike 3H3, CTX-471 did not induce significant peripheral immune cell activation or T-cell infiltration in the livers of treated mice, and doses as high as 100 mg/kg weekly were well tolerated in both mice and non-human primates with no signs of hepatic toxicity. Collectively, our data show that CTX-471 has a favorable and well-differentiated efficacy-safety profile that we attribute to a combination of its unique epitope, optimized affinity, and FcR-dependent activity. A Phase I clinical trial is now underway (NCT03881488) and actively recruiting patients.

Keywords: CD137, 4-1BB, Antibody, Translational.

## A141 / Combination cancer immunotherapy of anti-PD-1 antibody with recombinant adenovirus vector carrying p53 gene for urogenital cancers

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Urogenital cancers including kidney, bladder, and prostate cancers are common types of cancers worldwide especially in men. Although surgical intervention, chemotherapy, and/or hormonal therapy are standard of care for urogenital cancers, those approaches still need further improvements for efficacy and safety. The combination of cancer immunotherapy and gene therapy would be a promising approach in the era of Immuno-Oncology (I-O) [1].

Recently, a number of I-O drugs such as immune-checkpoint inhibitors and cytokine-armed oncolytic viruses have been approved for cancer treatments. However, mono- therapies with one I-O drug have shown the efficacy only in a small subset of patients. In particular, although gene therapy alone showed a local antitumor effect, it often failed to achieve a systemic anti-tumor activity in many clinical trials in the past. Therefore, combining gene therapy and I-O therapy is a promising approach to achieve the systemic antitumor activity.

In this study, we employed three murine urogenital cancer cell lines, TRAMP-C2 (prostate cancer), MBT-2 (bladder cancer) and Renca (renal cancer) cell lines. For in vitro study, these cell lines were infected with rAd-p53 [2] or rAd-LacZ (as a control virus) at multiple concentrations of Multiplicity of infection (MOI) and cultured for 6 days in vitro. As the results, rAd-p53 showed significantly higher cell cytotoxicity in TRAMP-C2 and Renca cells at 160 MOI compared with rAd-LacZ but not in MBT-2 cells. Also we examined the mRNA expressions of CAR (coxsackievirus and adenovirus receptor) in the three cell lines, and confirmed that MBT-2 cells expressed the significantly lower level of CAR mRNA compared to the other two cell lines. In addition, we determined the expression of programmed death ligand-1 (PD-L1) on those cell lines. The three cell lines were cultured in the presence of mouse IFN-γ at the concentration of 10 ng/mL with or without infection of rAd-p53 or rAd-LacZ at 40 MOI. After 3 days of culture, cells were stained with phycoerythrin-labeled anti-mouse PD-L1 antibody, and then PD-L1 expression was determined by flow cytometry. As the results, PD-L1 expression was increased in all cell lines by rAd-p53 infection. These results suggested that rAd-p53 might stimulate the PD-L1/PD-1 pathway and lead the T cell exhaustion. The combination of rAd-p53 and anti-PD-1 antibody could inhibit the T cell exhaustion and achieve the strong anti-tumor activity. In this presentation, we will also report the results of ongoing in vivo studies using prostate (TRAMP-C2) and kidney (Renca) murine tumor models.

Keywords: Immuno-Oncology, Adenoviral vector, p53, Urogenital cancers.

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## A142 / Effective targeting of tumor-specific HLA ligands with bispecific T cell-engaging receptor (TCER®) molecules

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T cell receptors (TCRs) naturally recognize human leukocyte antigen (HLA)-bound peptides derived from foreign and endogenous proteins regardless of their extracellular or intracellular location. Immunotherapy with bispecific, soluble TCRs has emerged as a novel and promising treatment modality for malignant diseases. Immatics has established the cutting-edge technology XCEPTOR® to discover and validate high avidity TCRs from the human T cell repertoire and, if required, further optimize the TCRs by affinity maturation. Bispecific T cell-engaging receptors (TCER®) are fusion proteins consisting of an affinity-maturated T cell receptor and a humanized T cell-recruiting antibody with an effector function-silenced IgG1 Fc part. Immatics is building a pipeline of highly potent TCER® molecules with an optimized bispecific design to confer extended half-life together with antibody-like stability and manufacturability characteristics. The molecular design allows for effective redirection of T cells towards target peptide-HLA selectively expressed in tumor tissues.

Here we present proof-of-concept data from a TCER® program targeting a cancer-testis antigen-derived peptide bound to HLA-A*02:01. We confirmed the abundant presence of the target peptide-HLA in several cancer indications and its absence in human normal tissues by using the XPRESIDENT® target discovery engine, which combines quantitative mass spectrometry, transcriptomics and bioinformatics. Yeast surface display technology was used to maturate the stability and affinity of a parental human TCR recognizing target peptide-HLA with high functional avidity and specificity. During maturation we applied XPRESIDENT®-guided off-target toxicity screening, incorporating the world's largest normal tissue immunopeptidome database, to deselect cross-reactive candidate TCRs.

The maturated TCRs were engineered into the TCER® scaffold and production in Chinese hamster ovary (CHO) cells generated highly stable molecules with low tendency for aggregation as confirmed during stress studies. Following TCR maturation, the TCER® molecules exhibited a 10, 000-fold increased binding affinity towards target peptide-HLA when compared to the parental TCR. The high affinity correlated with potent in vitro anti-tumor activity requiring only single-digit or double-digit picomolar concentrations of TCER® molecules to induce half-maximal lysis of tumor cells expressing the target peptide-HLA. Furthermore, using a tumor xenograft model in immunodeficient NOG mice, we could demonstrate complete remission of established tumors in all mice upon intravenous injection of TCER® molecules at a low dose of 0.05 mg/kg and twice weekly treatments for three weeks. We also examined the pharmacokinetic profile in NOG mice and determined a terminal half-life of TCER® molecules of more than 4 days, which presumably is compatible with a once weekly dosing regimen in patients. For the safety assessment of TCER® molecules, we measured killing of more than 20 different human normal tissue cell types derived from high risk organs. Notably, we could confirm a favorable safety window for selected TCER® molecules, which induced killing of normal tissue cells at a 1, 000-fold to 10, 000-fold higher concentration than required for killing of tumor cells. To further support safety of TCER® molecules, we also performed a comprehensive characterization of potential off-target peptides selected from the XPRESIDENT® normal tissue database based on its high similarity to the sequence of the target peptide or based on data from alternative screening approaches. The efficacy, safety and manufacturability data presented here provide preclinical proof-of-concept for our novel class, bispecific T cell-engaging receptor (TCER®) molecules for treatment of malignant diseases.

Keywords: T cell receptor, Bispecifics, TCER.

References:
Sarah Missel

## A143 / An RNA encoded extended half-life Interleukin-2 variant unfolds tumor immunity by substantial increase of the effector to Treg ratio

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Interleukin-2 (IL2) is a key cytokine in T-cell immunity. It supports the differentiation, proliferation, survival and effector functions of T cells. Recombinant IL2 was the first approved cancer immunotherapy and has been used for decades in the treatment of late stage malignant melanoma and renal cell cancer. Most patients with complete responses after IL2 treatment remain regression-free for more than 25 years after initial treatment, but overall response rates are low. Recombinant IL2 treatment is complicated by its very short half-life in the range of minutes and requirement of high and frequent dosing, which in turn potentiates side effects. Furthermore, a particular challenge of IL2 for cancer treatment is the preferential stimulation of Tregs in addition to activated tumor specific effector T cells already at low doses. Tregs can dampen anti-tumor immune responses and may counteract the beneficial effects of activated effector T cells.

We designed a nucleoside-modified RNA encoded IL2 variant with extended half-life, reduced Tregs bias and potent stimulation of human, cynomolgus or mouse effector T cells. Intravenously injected, lipid nanoparticle formulated RNA facilitated systemic as well as tumor and secondary lymphatic organ exposure for several days. In mice, a potent increase in CD8+ effector T cells and NK cell was observed while Tregs expansion was not detected. These findings translated into a significant anti-tumor effect in syngeneic mouse models. Therapeutic efficacy in mice was multiplied when combined with T-cell vaccination or anti-PD-1/PD-L1 checkpoint blockade. Patient recruitment for an open-label, clinical phase I/II, first-in-human trial is intended to start in the first half of 2020.

Keywords: RNA, Interleukin-2 variant.

## A144 / Cell penentrating peptides enhance neoantigen peptide vaccines

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Neoantigens are an important class of cancer antigen that are thought to mediate immune rejection of tumors, and which can be exploited by immunotherapy. However, neoantigen peptide vaccines to date have elicited weak immune responses in patients. We hypothesize that amphipathic cell penetrating peptides (CPPs) linked to neoantigen peptides will provide a simple and readily translatable approach for enhancing peptide vaccines. CPPs have been shown to deliver covalently attached peptides into the cytosol of cells and provide a method to bypass cross-presentation within antigen presenting cells. Additionally, their amphiphilic nature suggests increased interaction with serum proteins resulting in better lymph node trafficking. We conjugated known B16F10 antigens onto a library of CPPs and validated their ability to prime CD8+ T cells in vitro. The top performing CPPs were then tested for their ability to prime a T cell response in vivo. Antigen-specific T cell priming was greatly enhanced by the addition of CPPs when compared to free peptide immunization. Increased levels of IFNg and TNFa production in primed CD8+ T cells were also seen when neoantigens from the MC-38 adenocarcinoma model were conjugated to CPPs suggesting that the addition of an amphiphilic peptide to the end of antigens improves the in vivo

immune response toward that epitope. Therapeutically, these neoantigen-CPPs have elicited reduced tumor growth when used in combination with immune checkpoint blockade and increased overall survival. Parameters such as stability in the presence of serum, directed lymph node trafficking of antigens, uptake into a variety of antigen presenting cells, and increased the duration of antigen presentation contribute to the potency of these CPP-based vaccines. Understanding the mechanism by which CPPs enhance immune response provides an important foundation for future applications of neoantigen peptide vaccines.

Keywords: Cell penetrating peptides, Peptide vaccines, Neoantigens.

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# New targets and concepts

## A145 / Syngeneic mammary tumor models to investigate determinants of immunotherapy responsiveness

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The single-stranded DNA cytosine deaminase APOBEC3B is overexpressed and accounts for large proportions of somatic mutations in many cancer types1, 2. However, even in cancer types such as estrogen receptor-positive breast tumors where its mutagenic contribution often exceeds 10, 000 mutations3, the potential immunogenicity of APOBEC3B-derived non-synonymous mutations has yet to be investigated. We hypothesize that neoepitopes created by APOBEC3B-catalyzed mutagenesis will render breast tumors more responsive to immunotherapy. To test this idea we have developed a panel of syngeneic mammary tumor cell lines. Each cell line was derived from a different primary mammary tumor, expanded in culture, and demonstrated to re-engraft into fully immune-competent animals. Immune-profiling has shown surface expression of PD-L1 in the majority of lines. Experiments are ongoing to characterize APOBEC3B mutagenesis and evaluate immunotherapy responsiveness.

Keywords: APOBEC3B, Syngeneic Mouse Model, Breast Cancer, Immunotherapy.

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## A146 / A powerful ICOS agonist that enhances anti-tumor immune responses restored by immune checkpoint inhibitors

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The Inducible T cell Costimulator (ICOS, CD278) is a receptor in the CD28 family of B7-binding proteins expressed mostly by activated T cells. Upon binding to its ligand ICOS-L expressed on APCs, T cells are further activated by ICOS, resulting in their expansion and production of effector cytokines. The concept of targeting the ICOS: ICOS-L pathway in cancer immunotherapy, came from a 2014 study by James Allison's group, showing that tumor-bearing mice significantly benefited in terms of reduced tumor burden when their tumor cells expressed ICOS-L while being treated with a CTLA-4 blocking antibody [1]. As well, clinical data in patients treated with anti-CTLA-4 or anti-PD-1 mAbs have shown that the presence of ICOShi T cells correlated with an increased treatment response in these patients [2, 3]. Here, we report the design of a high-avidity ICOS agonist for enhancing restored anti-tumor responses using an engineered form of the extracellular Ig-like domain of ICOS-L, the natural ICOS ligand. We have recently observed that, higher-order, avidity-based engagement of other IgV-containing immune checkpoint receptors such as VISTA represents a key factor in agonizing signal transduction [4]. For instance, T cells cultured with dimeric Fc constructs of such ligands including VISTA are unable to agonize immune inhibitory signals in vitro unless such ligands are immobilized on a solid support [4]. We have thus recently designed a high avidity pentavalent form of the human ICOS-L extracellular domain by fusing it to a short α-helical peptide from the human cartilage oligomeric matrix protein (COMP). This ICOS agonist, termed ICOS-L.COMP, spontaneously assembles into stable pentamers, binds tightly to both human and murine ICOS (SPR; Kd <10 nM) and co-stimulates (with anti CD3 mAb) the proliferation and cytokine release of both murine and human CD4+ and CD8+ T cells when added as a soluble factor (unlike ICOS-L.Fc). Soluble ICOS-L.COMP also phosphorylates Akt in activated T cells and enhances the anti-tumor immune responses in vivo against MC38 or CT26 murine tu-

mors implanted in mice that had been restored using either anti-murine PD-1 or an anti-CTLA-4 mAb. Impressively, most mice treated with ICOS-L.COMP combined with an immune checkpoint inhibitor, were either cured or displayed a stable tumor burden in contrast to checkpoint monotherapies (tumor progression) or when ICOS-L.COMP was given as a monotherapy (comparable to a 'no treatment' group). Importantly, ICOS-L.COMP lacks an Fc domain thus eliminating Fc-associated off-target adverse effects. In summary, our results suggest that ICOS-L.COMP represents a new and powerful biologic to be used in combination with existing immune checkpoint inhibitors.

Keywords: ICOS, ICOS-L, ICOS agonist, High valency.

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## A147 / Ovarian carcinoma secretes arginase-1 containing small extracellular vesicles to suppress T-cell responses and to promote tumor growth

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Depletion of essential (L-tryptophan) or semi-essential (L-arginine) amino acids has been shown to suppress antitumor immune responses. Arginase-1 (Arg-1) is a cytosolic enzyme catalyzing degradation of L-arginine to L-ornithine and urea, depleting tumor microenvironment of this compound. T cells need arginine to support their proliferation in the lymph nodes and to promote their ability to kill tumor cells. Arginine deprivation is associated with decreased proliferation potential of activated T cells as well as with down-regulation of CD3 zeta, a major signal transducer from the T cell receptor (TCR). Thus, arginine deprivation due to increased Arg-1 activity is a very smart strategy of the tumor to avoid T cell-mediated effector mechanisms and, at the same time, one of the potential targets of anti-tumor therapy. Arg-1 is overexpressed not only by cancer-associated fibroblasts (CAFs), myeloid-derived suppressory cells (MDSCs) but also numerous cancer cells such as renal cell carcinoma, breast carcinoma, prostate cancer and colorectal cancer. We have recently discovered that small extracellular vesicles produced by ovarian cancer cells, contain enzymatically active Arg-1. Exosome-derived Arg-1 suppresses proliferation of CD4 and CD8-positive T cells activated with anti-CD3/anti-CD28 antibodies as well as T cells activated in the antigen-specific manner. All these in vitro effects are reversed by addition of an arginase inhibitor. Arg-1 containing tumor-derived exosomes are efficiently being engulfed by the dendritic cells and transported to the draining lymph nodes to create immunosuppressive environment at the site of the development of the immune response. Moreover, tumor-derived Arg-1 is detectable in the blood of ovarian cancer-bearing animals. Arg-1-expressing ovarian cancer grows faster in vivo and its growth is slowed down by the treatment of animals with the arginase inhibitor. In vivo, in Arg-1-expressing ovarian cancer cells arginase inhibition results in maturation of the peritoneal dendritic cells and their enhanced ability to engulf and present tumor-derived proteins. Altogether, our studies show that tumor cells use extracellular vesicles as vehicles to carry over long distances and deliver to immune cells a metabolic checkpoint molecule – ARG1, mitigating anti-tumor immune responses.

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Keywords: arginase, ovarian cancer, small extracellular vesicles, immune response.

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## A148 / Beyond mAbs: PRS-344/S095012, a 4-1BB / PD-L1 bispecific compound for tumor-localized immune cell activation

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With the expectation of decreasing immune related toxicities and improve overall response rate and survival in response to single ICP, Servier and Pieris are developing PRS-344/S095012. This bispecific molecule is based on the genetic fusion of an Anticalin protein moiety, obtained through Pieris Anticalin® technology, with an antibody moiety.

PRS-344/S095012 is a 4-1BB/PD-L1 bispecific that is designed to promote 4-1BB clustering by bridging 4-1BB-positive T cells with PD-L1. In relevant cell based assays, we show that PRS-344/S095012 is more potent than the combination of clinically relevant 4-1BB and PD-L1 benchmark antibodies. We report potent co-stimulatory T cell engagement of 4-1BB, in a PD-L1-dependent manner, in line with the desired MoA. This approach has the potential to provide a localized activation of the immune system with high efficacy and potentially reduced peripheral toxicity. Overall, our efficacy data provide proof of concept of PRS-344/S095012. Additionally, very good developability of PRS-344/S095012 supports IND-enabling studies of this promising compound.

Keywords: Immunotherapy, 4-1BB, PD-L1, bispecific.

## A149 / PD-L1 and ICOSL discriminate innate and adaptive dendritic cells in the head and neck cancer microenvironment

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Background: Three main maturation states have been described for dendritic cells (DC): activated, tolerogenic and homeostatic. In cancer, previous studies suggested that DC were in a tolerogenic state, but systematic phenotypic and functional studies in human tumors remain scarce. The objectives of our study were to determine the DC phenotypic and functional maturation states in head and neck squamous cell carcinomas (HNSCC).

Methods: For ex vivo analysis, we performed flow cytometry characterization of immune infiltrate of 22 untreated human HNSCC, and RNA sequencing of sorted tumor-infiltrating CD11c+HLA-DR+ subsets (n=6) and matched blood DC (n=3). The TMod database (Grandclaudon et al.), testing in vitro the effect of various stimuli on DC and subsequently on co-cultured T cells, was used to identify different DC maturation states and their potential for T cell modulation. Results: In a systematic in vitro study of 16 DC stimuli in over 154 individual observations with coupled sorted blood DC and CD4 T cell measurements, we identified two novel phenotypic maturation states delineated by the expression of PDL1 and ICOSL: (i) the "innate DC" phenotype, PDL1high and ICOSLlow/neg, in which cells were highly effective at secreting cytokines and chemokines but poorly induced CD4 T cell cytokines, and the "adaptive DC" phenotype, PDL1low and ICOSLhigh, poorly effective at secreting cytokines and chemokines, but strongly inducing CD4 T cell cytokines of all polarization profiles (Th1, Th2, Th9, Th17). In primary HNSCC tumors, the level of CD3 infiltration was positively associated with the level of DC infiltration and PDL1 expression on CD11c+HLA-DR+ cells, and negatively with the level of neutrophils and ICOSL expression on CD11c+HLA-DR+ cells. RNAseq analysis confirmed that DC purified from CD3high tumors overexpressed PDL1 and the other surface markers characteristic of innate DC, and not of adaptive DC, and that they overexpressed cytokines, chemokines, similarly to what we observed in our systematic in vitro study. Moreover, those tumor innate DC overexpressed maturation and migration markers, and potential targets, such as CD200, PVR and TREML1.

Conclusion: Our study revealed the existence of two novel DC maturation states with divergent functions, defining innate and adaptive DC, which may be identified by their expression of PD-L1 and ICOSL. CD3high HNSCC tumors were infiltrated with "innate DC" whereas CD3low tumors with immature DC (PDL1lowICOSLlow). We propose that immune stimuli respectively inducing innate or adaptive DC may be used as adjuvants in cancer immunotherapy, in combination with anti-PD-L1 when an innate DC phenotype is expected.

Keywords: Dendritic cells, Head and neck cancer, Tumor microenvironment, Cell phenotypes.

## A150 / Combined Rho-kinase inhibition and immunogenic cell death triggers and propagates immunity against cancer

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Activation of T-cell immune response is critical for the therapeutic efficacy of cancer immunotherapy. Current immunotherapies have shown remarkable clinical success against several cancers; however, significant responses remain restricted to a minority of patients. Here, we explored a therapeutic strategy that combines enhancing the phagocytic activity of antigen-presenting cells (APCs) with immunogenic cell death to trigger efficient antitumour immunity. We found that Rho-kinase (ROCK) blockade increases cancer cell phagocytosis and induces antitumour immunity through enhancement of T-cell priming by dendritic cells (DCs), leading to suppression of tumour growth in syngeneic tumour models. Combining ROCK blockade with immunogenic chemotherapy led to increased DC maturation and synergistic CD8+ cytotoxic T-cell priming and infiltration into tumours. This therapeutic strategy effectively suppressed tumour growth and improved overall survival in a genetic MMTV/Neu tumour model. Collectively, these results suggest that boosting intrinsic cancer immunity using immunogenic killing and enhanced phagocytosis is a promising therapeutic strategy for cancer immunotherapy.

Keywords: rho-kinase, immunogenic cell death, phagocytosis, dendritic cell.

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Gi-Hoon Nam, et al. Nat Comm. 2018;9(1):2165.

## A151 / Novel systems for robust screening of autoantigens in autoimmune disease

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T cells are the key effectors of cellular adaptive immune reactions and become activated when their T-cell receptor (TCR) engages an antigen presented on the surface of antigen-presenting cells (APCs). Newly generated T cells each express a single TCR from a theoretical repertoire size of 1018 unique receptors. To minimize the inherent potential for self-reactivity, the T-cell repertoire is carefully curated through tolerogenic processes. Indeed, failure to distinguish self from non-self can result in devastating autoimmune diseases. While the general mechanisms of immune tolerance and how their breakdown results in autoimmunity are now better understood, there is still only limited knowledge of the identity of the targeted antigens in many autoimmune diseases.

Here we propose a robust and versatile strategy to 'de-orphanize' patient-specific self-reactive TCRs, effectively uncovering the range of targeted antigens in any autoimmune disease of interest. We aim to generate a highly parallel system, capitalizing on patient-specific APCs, engineered to express tissue-specific antigens, and screen for potential activation of interacting T cells expressing patient-specific TCRs. We will first generate state-of-the-art donor-specific APC lines enabling expansion, molecular manipulation, and antigen presentation. Utilizing automated droplet-based microfluidic chambers, single APCs and T cells will be co-cultured and systematically screened for interacting dyads, resulting in T-cell activation. This will thus enable us to deconvolute the identity of disease-specific autoantigens, leading to the rational design of novel therapeutic strategies.

Keywords: Autoimmunity, target identification, Antigen, T cells.

## A152 / Serum amyloid A promotes inflammation-associated damage, macrophage infiltration and tumorigenesis in colitis-associated colon cancer

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The acute-phase protein Serum Amyloid A (SAA) has been shown to be involved in various inflammation-associated pathologies, and increased serum levels have been reported in several different types of cancers. Whether the increased serum levels are an indirect consequence of the chronic inflammation associated with cancer, or whether SAA has a direct influence on tumorigenesis, is currently still unclear.

In the current study we aimed to identify the role of SAA in colitis-associated cancer, a type of colon cancer associated with chronic inflammation experienced by patients with a history of inflammatory bowel disease.

We established a model of colitis-associated cancer in wild-type and SAA double knockout (SAADKO, knockout for SAA1 and SAA2) mice by following the azoxymethane/dextran sulfate sodium protocol, a well-established protocol that serves as an excellent representation of the disease in humans. Colon tissues and blood plasma collected from the mice model was used to assess inflammation, colitis-associated damage and tumorigenesis. In addition, the effect of both SAA knockdown and overexpressing colon cancer cell derived conditioned media on macrophages was assessed.

We observed increased colitis disease activity, colitis-associated tissue damage and macrophage infiltration in the colon of wild-type mice when compared to SAADKO mice. In addition, increased Ki-67 expression was also observed. Wild-type mice also displayed a higher tumor burden, characterized by increased proliferation, decreased apoptosis, and increased β-catenin activity when compared to SAADKO mice. SAA was also found to affect macrophage phenotype in vitro.

Based on these findings, we conclude that SAA has an active role in mediating colitis-associated symptoms and promoting tumorigenesis in colitis-associated cancer. Furthermore, we propose that SAA has the ability to attract and alter macrophage phenotype in the local tumor microenvironment, potentially promoting tumor growth. Identifying Methods to target and decrease or eliminate SAA levels could improve the quality of life and prognostic outcome of patients.

Keywords: Serum Amyloid A, Inflammation, Macrophage, Colitis.

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Sack, G. 2018. Serum Amyloid A – a review. Molecular Medicine, 24, 46. Thaker, A.I., Shaker, A., Rao, M.S. and Ciorba, M.A. 2012. Modeling Colitis-Associated Cancer with Azoxymethane (AOM) and Dextran Sulfate Sodium (DSS). Journal of Visualized Experiments, 67, 1-7.

## A153 / CDH1 and PTK2 genes significantly reflect therapeutic responses of overall survival to systemic therapy in patients with metastatic renal cell carcinoma

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Objectives: This study aimed to determine therapeutic responsive gene mutations after systemic first-line targeted therapy in patients with metastatic renal cell carcinoma (mRCC).

Methods: Between 2005 and 2017, 168 triplet-tissue block samples (with at least one tissue block having passed its quality checks) from 56 patients with mRCC were selected for targeted gene sequencing using the 88 targeted genes from the National Cancer Center, Korea kidney cancer panel. Overall survival (OS) was defined as the time interval between the diagnosis of metastasis and death. The patients were grouped into favorable (>12 months/>3 years), intermediate (3–12/12–36 months), and poor groups according to their progression-free survival (PFS)/OS (<3 months, <12 months) after first-line therapy. The therapeutic response was estimated to group into good response (CR+PR) and bad response (SD+PD) according to the RECIST v1.1. Groups (Favorable vs poor groups; and good vs bad groups) were compared in terms of their mutated genes. Statistical significance was assessed as p-values <0.05.

Results: The baseline characteristics were described table 1. Twenty-nine patients received only targeted therapy, and 27 patients received cytokine therapy and then targeted therapy. The median PFS and OS following first-line targeted therapy were 8.7 and 42 months, respectively. Memorial Sloan–Kettering Cancer Center and Heng risk criteria showed 28.9/65.8/5.3% and 26.3/57.9/15.8% for the favorable, intermediate, and poor risk groups, respectively. The first line therapeutic response showed 17.2%PR, 48.3%SD, and unknown 34.5%.

First comparative analysis of their mutated genes between two extreme survival groups, the poor (N=5 for PFS and N=7 for OS) and favorable (N=14 for PFS and N=20 for OS) groups showed the presence of either CDH1 (HR 4.217 for PFS and HR: 3.355 for OS) or PTK2 (HR: 3.458 for PFS), which significantly differentiated the PFS and OS in patients who received only targeted therapy (Log rank test: p<0.05). The MAPK14 (HR: 3.662 for PFS), FABP7 (HR: 5.856 for PFS, ) and STAG2 (HR:3.301 for PFS) were also significantly differentiated genes only for PFS(Log rank test: p<0.05, Figure 2 and 4). As for the patients who received cytokine and targeted therapy, only the presence of ATK3 gene mutations (HR: 8.67 for PFS) significantly differentiated PFS and OS (Log rank test p<0.05).

Second comparative analysis between CR+PR group vs SD+PD group showed mutated PTK2 gene was the worst significant gene for OS, whereas the non-mutated PTK2 gene showed the most favorable PFS (p<0.05, figure 3).

Last analysis about the cumulative effect of MAPK14, FABP7, STAG2, CDH1 and/or PTK2 on the PFS and OS showed The combination of the presence of either mutations also showed significant discriminating power for PFS and OS (p<0.05). Accumulation of all the five mutated gene had HR 15.555 comparing to the either one or none mutated five genes (p<0.05). Especially the combined PTHK2 and CHD1 mutated genes had worse HR 11.992 and HR 8.944 for OS than none mutated and one m, utated gene, respectively (p<0.05, figure 4).

Conclusion: CDH1 and PTK2 mutated gene were significant therapeutic responsive genes with worse prognostic outcomes for first-line targeted therapy.

Keywords: metastatic renal cell carcinoma, targeted gene, overall survival.

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## A154 / Macrophage scavenger receptor Clever-1 suppresses antitumor T cell responses by promoting the degradation of scavenged cargo

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Tumor-induced immunosuppression represents a significant obstacle to the successful treatment of cancer. Being foremost regulators of tumor-induced immunosuppression, tumor-associated macrophages have attracted significant interest as drug targets. Macrophages are remarkably adaptable cells that may reversibly polarize to either stimulate or suppress immune responses. Within tumors, macrophages often acquire an immunosuppressive phenotype that contributes to tumor progression. However, the clinical success of monotherapeutic macrophage depletion has been limited. An alternative approach to macrophage depletion is to exploit macrophage plasticity and "reeducate" suppressive macrophages to acquire an immunostimulatory phenotype that activates antitumor immunity. The scavenger receptor Clever-1, expressed by subsets of endothelial cells and macrophages, suppresses local and systemic antitumor immune responses. Recently, we reported that inhibiting the function of macrophage Clever-1 by genetic deletion or antibody-mediated interference reactivates endogenous tumor-reactive CD8+ T cells, significantly improving tumor control. Using macrophage-specific knockout mice, bone marrow chimeras and antibody-mediated cell depletion experiments, we demonstrated that the improved tumor control was mediated by CD8+ T cells but also required macrophages to occur. Genetic Clever-1 deletion rendered macrophages pheno-

typically more stimulatory and increased inflammatory cytokine levels. Still, the molecular mechanisms that render macrophages expressing Clever-1 immunosuppressive are not well understood. It is known that alongside dendritic cells, macrophages can also prime T cell responses. Macrophages are proficient at scavenging and degrading cargo from the extracellular space. For antigen cross-presentation to occur, however, the degradation rate of scavenged cargo has to be restricted. As a scavenger receptor, Clever-1 has various endogenous ligands, including oxidized and acetylated LDL and phosphatidylserine on cells undergoing apoptosis. After scavenging, Clever-1 transports its cargo to endosomes and phagosomes that eventually mature and fuse with lysosomes. Our preliminary data indicate that Clever-1 interacts with proton pumps regulating lysosomal acidification, a process required for efficient cargo degradation. Using flow cytometric Methods to analyze the uptake, acidification and degradation of scavenger receptor ligands, our unpublished results demonstrate that Clever-1 knockdown by RNA interference inhibits steady-state endosome maturation and the acidification and degradation of scavenged cargo. Mildly degradative environments facilitate antigen cross-presentation. Therefore, the rapid degradation of cargo scavenged by Clever-1 offers one putative explanation for the lack of an antitumor immune response in the presence of Clever-1-expressing macrophages. Hypothetically, Clever-1 blockade increases the cross-presentation of tumor antigens, thus potentially explaining the necessity of macrophages for CD8+ T cell activation. Taken together, this research further highlights the significance of macrophages in regulating T cell activity in the tumor microenvironment and the relevance of macrophage reeducation as a means of overcoming tumor-induced immunosuppression.

Keywords: tumor-associated macrophages, scavenger receptor, immunosuppression, antigen degradation.

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Viitala, M. K. et al. Immunotherapeutic blockade of macrophage Clever-1 reactivates the CD8+ T-cell response against immunosuppressive tumors. Clinical Cancer Research 25, 3289–3303 (2019).

## A155 / DNA-dependent protein kinase initiates a STING-independent DNA sensing pathway in human cells

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Novel cancer immunotherapies that specifically activate innate antiviral pathways within tumors are currently being pursued by a myriad of pharmaceutical companies. Most of these efforts rely on agonism of cGAS or STING, which have been established as key components of foreign DNA sensing in cells. Intriguingly, we have discovered a novel STING-independent DNA sensing pathway (SIDSP) in human cells that could potentially be harnessed to enhance immune responses to cancer. In short, we found that DNA-dependent protein kinase (DNA-PK) recognizes free ends of DNA and leads to downstream phosphorylation of IRF3 and production of interferons and interferon-stimulated genes. We also discovered that the heat shock protein A8 (HSPA8) is phosphorylated downstream of DNA-PK and acts as a unique marker for the SIDSP. However, the detailed mechanism of this pathway has yet to be elucidated. Although DNA-PK is a crucial component of the non-homologous end joining pathway activated by double-stranded DNA breaks, we were surprised to find that DNA damaging agents did not activate the SIDSP. Therefore, we hypothesize that the downstream effectors/targets of DNA-PK are different with regard to its two "modalities" – DNA repair and the antiviral response. To identify unique components of the antiviral modality of the DNA-PK SIDSP upstream of IRF3 phosphorylation, we have performed discovery phosphoproteomics analysis on DNA-transfected cells. Importantly, we confirmed that HSPA8 phosphopeptides increase after DNA transfection and diminish upon treatment with the specific DNA-PK inhibitor Nu7441. We have identified phosphoproteins canonically involved in DNA damage pathways that were activated in response to DNA transfection, and are currently following up on whether these hits are related to SIDSP signaling or whether DNA transfection is causing other DNA damage responses in the cell. We are also performing comparative phosphoproteomics experiments to identify additional unique components of the SIDSP that, like HSPA8 phosphorylation, are not activated by DNA damage. Overall, we aim to illuminate as-of-yet unknown players in this novel pathway in order to design specific SIDSP agonists for use in potentiating cancer immunotherapy.

Keywords: cGAS/STING, nucleic acid sensing, type I interferon, DNA-PK.

## A156 / Intraepithelial lymphocytes undertake skin immunosurveillance by sensing Skint1

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Intraepithelial lymphocytes (IEL) expressing unconventional T cell receptor (TCR) repertoires of limited diversity dominate epithelial surfaces where they intimately associate with epithelial cells and can suppress malignancy[1]. We have previously shown that epithelium-restricted members of the Btnl family, related to PDL1 and B7, direct the maturation of γδ IEL in a tissue- and TCR-specific manner[2]. For example, Btnl family molecules Skint1 and Skint2 positively select the thymic progenitors of Vγ5Vδ1+ skin IEL[3]. Nonetheless, the role(s) played by extra-thymic Skint1, expressed at steady-state by keratinocytes, is unresolved. Moreover, while mature epidermal IEL promote skin integrity and suppress carcinogenesis, the contribution(s) to that of their invariant TCR is undetermined[4, 5]. Here we show that Skint1 mediates constitutive Vγ5Vδ1 TCR sensing of keratinocytes, suggesting that keratinocytes actively signal the normal state of the tissue to IEL. Indeed, long-term inhibition of Skint1-IEL crosstalk resulted in abnormal IEL activation and skin inflammation. To study natural IEL responses to dysregulated epithelium, we examined ultraviolet radiation (UVR) of the skin, to which local IEL responded by upregulating the TCR signalling reporter Nur77 and a signature of cytotoxic and immunomodulatory genes. Strikingly, acute disruption of Skint1-IEL interaction prevented Nur77 induction and cytotoxic gene expression by IEL following UVR stress. Furthermore, inhibition or genetic deficiency of Skint1 resulted in exacerbated skin inflammation following UVR. Collectively, these data suggest that IEL constitutively sense keratinocyte Skint1 via their invariant Vγ5Vδ1 TCR, which regulates their homeostasis while also maintaining their competence to respond to epithelial stress, possibly by sustaining sensitivity to activation by co-stimulatory signals and/or cytokines. This modus operandi of IEL immunosurveillance may be highly relevant to human cancers, given the evident conservation of BTNL-IEL regulation in the human colon.

Keywords: immunosurveillance, unconventional lymphocytes, T cell receptor, epithelium.

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## A157 / Polycomb repressive complex 2 represses pro-fibrotic activity in the decidual stroma by modulating the transforming growth factor β pathway.

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The stromal environment of an organ can influence how that organ reacts to injury and infection, its predisposition to fibrotic transformations, and its ability to regulate leukocyte trafficking to and from inflamed sites to mediate tissue repair. Importantly, growing tumors interact with their surrounding stroma and incorporate it into their own environment. To accomplish this, the tumor co-opts the natural wound healing responses of the surrounding tissue, generating a chronic pro-fibrotic wounding state [1]. This leads to the induction and accumulation of cancer-associated fibroblasts, which have been demonstrated to play a complex role in tumor biology, contributing to the metastatic potential of a cancer and mediating local immunoregulation [2].

The decidual stroma is generated from the uterine endometrium upon embryo implantation and provides support for the developing fetus. Because of its specialized and fascinating functions, as well as its tightly regulated and stereotypical development, it provides an excellent model to study stromal biology. Much like the tumor stroma, the maternal decidua supports the growth and invasion of foreign tissue (in this case, the fetus and invading placenta) and protects the fetus from attack by the maternal immune system. In contrast to the endometrium of the non-pregnant uterus, the decidua not only prevents the egress of maternal dendritic cells from the maternal-fetal interface, but also blocks the influx of effector T cells, including ones that might be generated by systemic pathogens. However, unlike the tumor stroma, the decidua does not accumulate activated fibroblasts and does not mount robust wound healing responses. Recent work from our lab has uncovered that transformation of the endometrium into the decidua triggers an epigenetic silencing program within decidual stromal cells that transcriptionally silences a diverse set of ~800 genes, including genes associated with both type 1 immunity, as well as fibroblast activation and wound healing [3]. This program is generated by PRC2 (Polycomb Repressive Complex 2), whose primary catalytic subunit is the histone methyltransferase EZH2 (Enhancer of Zeste Homolog 2). We employed a mouse model in which Ezh2 has been conditionally deleted within the uterus to determine how deficient PRC2 activity alters the capacity of the decidua to mount wound healing responses and regulate leukocyte trafficking at the maternal-fetal interface. We discovered that the decidua employs PRC2-mediated gene silencing to tightly control local immune regulation and pro-fibrotic signals. This may serve to prevent the decidua from transforming into a chronically wounded cancer stroma-like state in response to invasion and growth of fetal tissues. Transforming growth factor β (TGF-β) signaling was central to the observed defects, and in-vivo blockade of TGF-β signaling partially rescued the dysregulation caused by PRC2 deficiency. Our investigation stands at the novel crossroads of immunity, fibrotic responses, and epigenetics, and the mechanisms we uncover will give us greater insight into stromal control of its local environment.

Keywords: Epigenetics, Fibrosis, Tumor Microenvironment, Pregnancy Immunology.

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## A158 / Pembrolizumab interferes with the differentiation of human FoxP3+ induced Tregs, but not with FoxP3 stability, through activation of mTOR and STAT1

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Programmed cell death 1 (PD-1) is critical for T regulatory cells (Tregs) to maintain peripheral tolerance to self-antigens. In the tumor microenvironment, interaction between PD-1 and its ligands supports tumor immune evasion. Pembrolizumab blocks interactions of PD-1 with its ligands, enhancing anti-tumor and clinical responses. We and others have reported that pembrolizumab does not affect function or phenotype of thymic-derived Tregs (tTreg); however, little is known about its effect on extrathymic differentiation of peripheral Tregs (pTregs). In this study, we investigated the effect of pembrolizumab on in vitro induced Tregs (iTregs). Our work showed that PD-1 blockade interferes with iTreg differentiation, and has no potential effect on the stability of FoxP3 after differentiation. Different Methods including transcriptomic analyses confirmed that this effect was mediated by activating mTOR and STAT-1 and inhibiting MAPK pathways, shifting the iTreg polarization in favor of Th1 and Th17 subsets. To confirm the role of mTOR activation, we found that rapamycin diminished the effect of pembrolizumab-mediated downregulation of FoxP3. Ingenuity pathway analysis revealed that pembrolizumab-treated iTregs showed upregulation of genes promoting DNA repair and immune cell trafficking, in addition to downregulation of genes supporting cellular assembly and organization. This is the first study to show that pembrolizumab can downregulate human FoxP3 in iTregs, and to disclose some of the molecular pathways involved.

Keywords: PD-1, human iTreg, Keytruda.

## A159 / Intracellular delivery of nucleic acids by hapten-binding bispecific antibodies

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Here, we describe a delivery system consisting of two to three entities: hapten-binding bispecific antibodies (bsAbs), haptenylated nucleic acid-binding cell penetrating peptides (CPPs), and nucleic acids as the payload. The combined modules not only accomplish a cell-specific targeting but also deliver nucleic acids across biological membranes and into the nucleus. bsAbs, as one segment of the platform, recognize the cell surface antigens of the target cells as well as haptens thus enabling the targeted delivery of the payload. Haptenylated nucleic acid-binding CPPs that are derived from human proteins can be used as a bridge of antibodies to the payload. The payload coupled to the targeting moiety can vary from small molecules to even considerably larger molecules as plasmids. Here, the wrapping of plasmids by histones to plasmid-chromatin packages and the additional bridging by CPPs to bsAbs allows an efficient payload delivery to the therapeutic relevant intracellular space.

The advantages of the described delivery platform are manifold: the entities are human-derived thus circumventing potential immunogenicity concerns, the pharmacokinetic properties of small compounds are modulated, the payload delivery is targeted, and a high efficiency is maintained.

Keywords: nucleic acid delivery, bispecific antibodies.

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Killian T, Buntz A, Herlet T, Seul H, Mundigl O, Längst G, Brinkmann U. Antibody-targeted chromatin enables effective intracellular delivery and functionality of CRISPR/Cas9 expression plasmids. Nucleic Acids Res. 2019

## A160 / Mechanisms whereby liquid phase separation of cGAS activates innate immune signaling

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Type I Interferon (IFN) response is a key player in eliciting anti-tumor immunity[1]. Type I IFNs activate the T-cell response to cancer and can prevent tumor growth from within tumor cells by inducing senescence and cell death[2]. Cyclic GMP-AMP synthase (cGAS) is an important control point anti-tumor type I IFN signaling. Tumor DNA that is mislocalized to the cytoplasm stimulates cGAS enzymatic activity, triggering a signal cascade that culminates in the transcription of type I IFNs and other cytokines[3]. Because the cGAS enzyme plays a critical role in tumor immunosurveillance, there has been considerable interest in understanding the molecular details of its activation and activity. It was recently reported that in the presence of DNA, cGAS undergoes liquid-liquid phase separation and that this process dramatically increases its specific activity, but the mechanism of this effect is unclear[4]. Here, we present ongoing work to addresses the hypothesis that phase separation promotes cGAS signaling by creating a microenvironment that facilitates cGAS activity. The studies of cGAS phase separation presented here will provide a better understanding of the molecular basis of cGAS activation and may identify new strategies and reagents to improve the efficacy of cancer immunotherapy.

Keywords: cGAS, liquid-liquid phase separation.

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## A161 / CARs, TRUCKs, and beyond : the next generation CAR T cells

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Adoptive therapy with chimeric antigen receptor (CAR) redirected T cells achieved spectacular remissions of refractory leukemia/lymphoma, the treatment of solid tumors remains so far challenging. In new developments, CAR T cells are used as "living factories" to deposit immune modulating cytokines in the targeted tumor tissue aiming at converting the immune cell environment into a more favorite one to sustain a productive anti-tumor response. Such TRUCKs (T cells redirected for unrestricted cytokine release and killing) releasing IL-12 or IL-18 upon CAR engagement of antigen in the CAR targeted tumor lesion are superior in attracting and activating the innate immune response in the tumor lesion. In a further development a blocking anti-CD30 antibody is integrated into the extracellular CAR domain to prevent CD30L engagement. T cells engineered with an anti-CEA and CD30 blocking CAR showed an improved response against CEA+ CD30-negative solid tumors. This new CAR design aims at targeting tumor cells by one scFv and blocking the CD30/CD30L interaction on the T cell by the other scFv. The strategy thereby combines tumor targeting with preventing repression in order to prolong the anti-tumor response.

Keywords : CAR, TRUCK, CD30.

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## A162 / SLC19A1 transports immunoreactive cyclic dinucleotides

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The accumulation of DNA in the cytosol serves as a key immunostimulatory signal associated with infections, cancer and genomic damage. Cytosolic DNA triggers immune responses by activating the cGAS/STING pathway. The binding of DNA to the cytosolic enzyme cGAMP synthase (cGAS), activates its enzymatic activity, leading to the synthesis of a second messenger, cyclic[G(2', 5') pA(3', 5')] (2'3'-cGAMP). 2'3'-cGAMP, a cyclic dinucleotide (CDN), activates the protein 'stimulator of interferon genes' (STING), which in turn activates the transcription factors IRF3 and NF-κB promoting the transcription of genes encoding type I interferons and other cytokines and mediators that stimulate a broader immune response. Exogenous 2'3'-cGAMP and other CDNs, including CDNs produced by bacteria and synthetic CDNs used in cancer immunotherapy, must traverse the cell membrane to activate STING in target cells. How these charged CDNs pass through the lipid bilayer is unknown. Here we used a genome-wide CRISPR interference screen to identify the reduced folate carrier SLC19A1 as the major transporter for CDNs. CDN uptake and functional responses are inhibited by depleting SLC19A1 from human cells and enhanced by overexpressing SLC19A1. In both human cell lines and primary cells ex vivo, CDN uptake is inhibited by folate and blocked by the SLC19A1 inhibitor sulfasalazine and the antifolate methotrexate, medications approved for the treatment of inflammatory diseases. The identification of SLC19A1 as the major transporter of CDNs into cells has implications for the immunotherapeutic treatment of cancer, host responsiveness to CDN-producing pathogenic microorganisms, and potentially in certain inflammatory diseases.

Keywords : Immunotherapy, Innate Immunity, Signal Transduction.

## A163 / Nanobody-guided delivery augments cell surface protein ligand potency and selectivity

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Conjugates of antibodies and bioactive compounds, such as antibody-drug conjugates (ADCs), hold great promise for targeted delivery of therapeutic compounds to cell types or anatomical structures of choice by virtue of the selectivity engendered by the antibody. However, ADCs have not yet realized widespread success due in part to complications in producing homogeneous conjugates and inefficient delivery of drug payloads to the site of action at cells. We developed a new type of ADC consisting of antibody fragments amenable to site-specific modification with Sortase A and ligands that bind to cell surface receptors, with a specific focus on ligands for G protein coupled receptors (GPCRs). We target parathyroid hormone receptor-1, which is the target of drugs approved to treat osteoporosis. In our system we augment the signaling activity and specificity of suboptimal N terminal peptide fragments of parathyroid hormone by conjugating them to antibody fragments (variable domains of heavy chain only antibodies, VHH) that bind to the receptor extracellular domain. We show that these conjugates exhibit biological activity superior to the N terminal fragment peptide on cells and in mice. The lead conjugate also shows higher selectivity between receptor subtypes than an analogue of a human osteoporosis drug. The platform described here provides a new approach for generating ligands for cell surface receptors with properties superior to those provided by nature or chemists alone. This approach may be useful for selectively inducing immune responses in a desired subset of cells.

Keywords : Antibody, Receptor, Targeted delivery.

## A164 / Chromatin destabilization by CBL0137 and panobinostat leads to robust interferon response and disease regression in high-risk childhood cancer models

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Despite impressive progress within the last few decades, childhood cancer is still the most common cause of death by disease in children. Moreover, surviving patients, especially young children, often experience severe long-term side effects caused by high-dose cytotoxic drugs and radiation. Developing more effective and safer therapies for childhood cancer is therefore urgently warranted. CBL0137, an analogue of the antimalarial agent quinacrine, is a safe, non-DNA damaging novel anticancer drug that is currently in phase I trials for adult refractory and relapsed cancers. By distorting the structure of DNA, CBL0137 destabilizes nucleosomes and traps histone chaperone FACT into the chromatin, thereby simultaneously modulating a number of anti-cancer mechanisms including p53 pathway activation, NFkB inhibition, inhibition of FACT-mediated DNA repair, and activation of an IFN response. Based on our preclinical studies showing the safety and efficacy of CBL0137 in animal models of high-risk childhood cancers with poor outcome, namely neuroblastoma, MLL-rearranged

leukemia and DIPG, the compound is now also being progressed into a phase I trial in children. Here, we have identified that the FDA-approved histone deacetylase (HDAC) inhibitor, panobinostat, which affects the structure of chromatin by epigenetic modifications, acts as a strong potentiator of CBL0137 to reduce cell viability and clonogenicity in neuroblastoma, DIPG and MLL-rearranged leukemia in vitro and delay cancer progression and increase survival in xenograft models in vivo. More strikingly, the combination eradicated established neuroblastoma in 100% of Th-MYCN transgenic neuroblastoma mice tested, which is the most significant result we have obtained in this aggressive, immunocompetent neuroblastoma model. The combination elicited a rapid and robust interferon response in the tumor microenvironment, significantly increasing expression of interferon-induced genes, such as Ifit3b and Cxcl10 in non-tumor cells, compared to either single agent. In addition, both circulating and tumor-specific CD4+ T cells were significantly increased in the immunocompetent model, strongly indicating that CBL0137/panobinostat may activate anti-tumor immunity in immunocompetent settings. In vitro mechanistic studies showed that panobinostat enhanced chromatin destabilization induced by CBL0137, resulting in histone eviction and DNA repair suppression. Live-cell imaging of mCherry-H2B tagged tumor cells revealed that the combination produced a nuclear-blebbing phenotype possibility due to nuclear membrane rapture, a process that can elicit interferon response through releasing of genomic DNA into the cytosol. Our studies have identified CBL0137 and panobinostat as a highly effective drug combination for neuroblastoma and other aggressive pediatric malignancies. This combination likely halts tumor growth through a two-pronged attack: enhanced chromatin destabilization followed by activation of an interferon response and heightened anti-tumor immunity. Our results will greatly facilitate clinical development of effective and non-toxic therapies for childhood cancer.

Keywords: Interferon, Chromatin, HDAC inhibitor, Innate immunity.

## A165 / DUSP22 ablation enhances lung tumorigenesis and tumor-mediated immunosuppression

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DUSP22, a member of the dual-specificity phosphatases (DUSPs) family, is a key phosphatase controlling the activity of protein kinases and transcription factors through dephosphorylation. DUSP22 knockout mice manifest multi-organ inflammation, supporting a role for DUSP22 in maintaining immune homeostasis; however, little is known about the in vivo role of DUSP22 during tumor development. We performed bioinformatics analysis on public available TCGA lung adenocarcinoma datasets according to DUSP22 expression levels in patients, and found that patients with lower DUSP22 expression levels are more likely to have poor survival. In addition, the expression levels of cell cycle-related genes, identified as poor prognostic markers of various cancers, are negatively correlated with DUSP22 expression, which suggests a negative role for DUSP22 in controlling lung tumorigenesis. By utilizing a murine lung tumor model driven by the combination of mutated EGFR amplification (exon 19 deletion, EGFR-del) and the loss of DUSP22, we found that genetic ablation of DUSP22 promoted EGFR-del-mediated lung tumor development. Analysis of the lungs from EGFR-del/DUSP22-/- mice showed that DUSP22 ablation enhanced EGFR RTK phosphorylation levels (Tyr1068), decreased interferon-γ levels, and decreased tumor-infiltrating CD8 T cell population, showing phenotypic features associated with accelerating lung tumor progression and the immunosuppressive landscape. Consistently, knockdown of DUSP22 expression in human and murine syngeneic lung tumor cells reduced the proliferation or activation of T cells during in vitro co-culture, suggesting that DUSP22-controlled tumor-intrinsic signaling is crucial for tumor-mediated immunosuppression. Currently, we are analyzing gene expression profiles of lung cancer cells by RNA-sequencing (control vs. DUSP22 knockdown) to identify potential molecules that could be responsible for immunosuppression. Meanwhile, we are testing several immunosuppressive molecules by employing antibody blockade. Collectively, our current data indicate that DUSP22 under-expression or loss of DUSP22 function in lung cancer cells may facilitate to establish an immunosuppressive tumor microenvironment for enhancing EGFR-del-mediated lung tumorigenesis.

Keywords: DUSP22, lung adenocarcinoma, immunosuppression.

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## A166 / Role of the local innervation in the formation of bronchus-associated Tertiary Lymphoïd Structures

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The immune system plays a key role in tumor control and development. My laboratory has shown in a pioneering work that i) lymph node (LN)-like structures called tertiary lymphoid structures (TLS) are present in lung tumors, and ii) their presence is associated with a favorable prognosis in non-small-cell lung cancer (NSCLC). Recent results of the laboratory have also indicated that TLS present in lung tumors are critical sites of development of cellular and humoral anti-tumor responses. Interestingly, LN and TLS have a similar, albeit not identical, pattern of development. However, whether the peripheral nervous system is involved in TLS neogenesis as it has been suggested for LN is unknown. Thus, I set up a murine model of lung inflammation where TLS can be induced and their kinetics of maintenance followed-up. Thanks to this model, we could demonstrate that an in vivo chemically-induced specific denervation of sympathetic nervous fibers has a strong impact on TLS formation in inflamed lungs. Using a 2nd generation of multiplex 3D-stainings for imaging both nerve fibers and TLS in the whole lung, as well as other Methods, we observed that the number of TLS drops dramatically in the mice as compared to control mice. The analysis of the immune cell composition of chemically-treated mice is currently investigated to determine which immune population(s) is(are) impacted by the systemic denervation. We will now decipher the molecular and cellular mechanisms that are responsible for the cross-talk between the sympathetic nervous fibers and the TLS present in the inflamed lung.

Keywords: Tertiary Lymphoïd Structure, neogenesis, peripheral nervous system.

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## A167 / Targeting tumor-associated TREM1+ inhibitory myeloid cells to promote anti-tumor immunity

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The tumor microenvironment (TME) contains diverse types of myeloid cells, including tumor-associated macrophages (TAMs), tumor associated myeloid-derived suppressor cells (MDSCs), and tumor-associated neutrophils (TANs). TAMs and TANs exhibit a spectrum of functional phenotypes ranging from immunosuppressive M2-like macrophages or N2-like neutrophils that promote tumor growth to pro-inflammatory M1-like macrophages and N1-like neutrophils that promote anti-tumor immunity. Therapies that shift the balance of inhibitory myeloid cells towards a more pro-inflammatory phenotype are expected to positively impact anti-tumor immune responses and convert checkpoint inhibitor (CPI)-resistant tumors into CPI-sensitive tumors. To target TAMs, TANs, and MDSCs, we developed anti-human and anti-mouse TREM1 mAbs, termed PY159 and PY159m, respectively. These mAbs induced downstream signaling pathways that promoted production of a highly selective subset of pro-inflammatory factors in ex vivo assays. Additionally, PY159 treatment increased the expression of HLA-DR and the costimulatory molecule, CD40, which play critical roles in antigen presentation and T cell activation. Molecular profiling of syngeneic tumors from mice treated with PY159m demonstrated activation of both innate and adaptive immune pathways consistent with the ex vivo assay results. These findings suggest that anti-TREM1 therapy re-educates TREM1+ myeloid cell into pro-inflammatory cells. In vivo, single agent treatment using PY159m had anti-tumor activity in a number of syngeneic tumor models. Particularly striking, PY159m in combination with anti-PD-1 converted anti-PD-1 resistant tumors into treatment-sensitive tumors, underscoring the utility of targeting intratumor TREM1+ TAMs, TANs, and mMDSCs. Mice cured of their tumors by PY159m combination therapy were resistant to tumor re-challenge demonstrating that targeting myeloid cells supports adaptive immunity and induces long-term immunological memory. A survey of the immune infiltrates by both flow cytometry and RNA expression in a variety of human solid tumors showed that TREM1+ myeloid cells were present at a high frequency in all tested tumors. Furthermore, for a number of tumor types there was an inverse correlation between high levels of TREM1 expression and patient survival probability. Based on these preclinical findings, we are developing PY159 as a therapeutic agent for monotherapy and/or CPI combination therapy for solid tumors.

Keywords: Suppressive myeloid cells, Anti-tumor immunity, Myeloid tuning, CPI combination therapy.

## A168 / Mapping γδT cell genetic networks

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γδ T cells efficiently detect and kill cancer cells. γδ T cell clones exhibit specificity against a wide array of cancer cell lines. However, this cell type remains one of the least understood aspects of the immune system. Specifically, we have a poor grasp on γδ T cells' genetic wiring that controls their activation, cytotoxic potential, and cytokine secretion in the presence of cancer cells. Applying functional genomics and genome-wide CRISPR knockout screens in primary human γδ T cells, we are generating unbiased maps of intracellular signaling that regulate γδ T cells. Using single guide RNA (sgRNA) lentiviral infection with Cas9 protein electroporation (SLICE), a method recently developed in the Marson Laboratory, we are able to perform genome-wide CRISPR screens in primary T cells. This approach will be expanded to testing the hypothesized distinction between adaptive (Vδ1) and innate (Vδ9Vδ2) human γδ T cells. These studies will uncover novel γδ T cell genetic networks, thereby creating new therapeutic avenues in cancer immunology.

## A169 / Immuno-oncology therapy with HERA-GITRL: The novel hexavalent human GITR agonist that induces T cell-mediated anti-tumor immune responses and shows superior activity in direct comParison to benchmark agonistic antibodies

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Glucocorticoid-induced TNF-R-related protein (TNFRSF18, GITR, CD357), expressed by T cells, upon binding its ligand (TNFSF18, GITRL), primarily expressed by myeloid cells, provides co-stimulatory signals that enhance T cell activity. Due to the important role that TNF Receptor Superfamily (TNF-R-SF) members, including GITR, play in regulating immune functions, they have been key immunotherapeutic targets for over 20 years.

The limited clinical efficacy of antibody-based GITR agonists results from structural and functional characteristics of antibodies, including the presence of only two target-binding domains per molecule, that are unsuitable for stimulating the members of the TNF-R-SF. TNF-R-SF signaling is a structurally well-defined event that requires trimeric receptor clustering with proper spatial orientation. While the ligands naturally exist as trivalent functional units, the receptors are separated on the cell surface and need to be organized into functional trimeric assemblies. Multiple clusters of trimeric receptor assemblies are necessary to induce proper intracellular domain organization, which is a prerequisite for signaling.

To overcome the inadequacies of bivalent antibodies, we have developed HERA-GITRL, a fully human hexavalent TNF receptor agonist (HERA) targeting GITR and mimicking the natural signaling concept. HERA-GITRL is composed of three GITRL-receptor binding domains in a single chain arrangement fused to a silenced human IgG1 Fc-domain, which serves as a dimerization scaffold. This generates a hexavalent molecule that mimics the natural ligand and enables efficient receptor recruitment with optimal stoichiometry. Because of the unique design of the silenced Fc-domain, HERA-GITRL allows the study of pure GITR agonism in contrast to Fc-mediated mixed modes of action employed by agonistic antibodies. Here we report in vitro and in vivo properties of our novel HERA-GITRL construct.

For functional characterization of HERA-GITRL in vitro, human T cells were isolated from healthy-donor blood and stimulated with anti-CD3 antibody in the presence of HERA-GITRL. Consistently, HERA-GITRL increased the activity of T cells, including proliferation, differentiation and cytokine production, even in the presence of regulatory T cells. Importantly, in the absence of T cell receptor stimulation, HERA-GITRL has no effect on T cell activity.

In line with these findings, mmHERA-GITRL enhanced antigen-specific clonal expansion of both CD4+ (OT-II) and CD8+ (OT-I) T cells in vivo while having no effect on non-specific T cells. In addition, mmHERA-GITRL showed single-agent anti-tumor activity in two subcutaneous syngeneic colon cancer models (CT26wt and MC38-CEA) on two different genetic Backgrounds. Importantly, this activity is independent of its Fc gamma receptor-binding functionality, as both mmHERA-GITRL with a functional Fc- and a silenced Fc-domain showed similar tumor growth inhibition and T cell activity. Finally, in a direct in vitro comParison to a bivalent clinical benchmark anti-GITR antibody and a trivalent GITRL, only the hexavalent HERA-GITRL showed full biological activity independent of additional crosslinking. Interestingly, while HERA-GITRL consistently increased T cell activity, the clinical benchmark anti-GITR antibody consistently reduced the T cell response.

In summary, here we describe the development of HERA-GITRL, a true GITR agonist with a clearly defined mechanism of action. By

clustering six receptor chains in a spatially well-defined manner, HERA-GITRL induces potent agonistic activity without being dependent on additional Fc gamma receptor-mediated crosslinking.

Keywords: GITR, CD357, single-chain GITRL, TNF-SF.

## A170 / Promotion of immune responses by using pHLIP technology to target tumor cell surface acidity

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pH-Low Insertion Peptides (pHLIP® peptides) are a platform technology that uses surface acidity to target individual cells within tumors. If a cell surface is acidic, a pHLIP® will fold and insert to form a stable helix by inserting its C-terminus across the membrane into the cytoplasm and leaving its N-terminus in the extracellular space. Within the tumor microenvironment, pHLIPs® target cancer cells and tumor associated macrophages (TAMs) very well. In the uses of traditional targeting using molecular biomarkers, resistance by clonal selection often emerges. pHLIP® overcomes the serious issues of tumor heterogeneity and clonal selection, since pHLIP® targets a universal characteristic of tumor cells – a high cell surface acidity. Extracellular acidosis promotes tumor development, progression and invasiveness. A combination of metabolic effects acidifies tumor cell interiors, so, to maintain intracellular pH, cells pump out lactic acid and protons, acidifying the extracellular space. In addition, the overexpression of carbonic anhydrases on the surfaces of cancer cells and the electrochemical potential further contribute to an acidification of the environment, especially near the cell surface. We have established that the pH near the surface of a cancer cell can be 0.5-0.7 pH units lower than the bulk extracellular pH, and demonstrated that the surface pH is sensitive to cell glycolytic activity. An individual tumor cell maintains acidity near its surface and can be detected even in well-perfused areas.

Using the insertion of pHLIP®, various diagnostic and therapeutic cargo molecules can be located at cell surfaces and/or directly delivered into their cytoplasm. Since the N-terminus of an inserted pHLIP remains at the cell surface, it can be used to position imaging probes or immuno-stimulating agents at tumor cell surfaces. We are translating pHLIP®-based imaging agents to the clinic for diagnostic PET imaging, fluorescence-guided surgical procedures and photo-acoustic pre-operative imaging. Similarly, immunostimulatory agents can be positioned on tumor cell surfaces by pHLIP®, including antigens, carbohydrates, cytokines, chemokines and proteins.

Another use of pHLIP® is to use attachment of a therapeutic cargo to the inserting C-terminus, releasing it into the cell via a cleavable link. The pHLIP® intracellular delivery mechanism bypasses endosomal uptake and assures direct intracellular delivery. Molecules delivered into the cytoplasm can be polar (Log P in the range of -0.5 to -1.5) and large (4-5 kDa unstructured polymers and small proteins), as well as moderately hydrophobic (drugs and drug-like molecules) and small (< 1 kDa). Among moderately hydrophobic molecules targeted and delivered in vivo to tumor cells are PARP and tubulin inhibitors, DNA binding molecules, and other disruptive agents. Among polar molecules delivered by pHLIP® into cancer cells and TAMs are polar toxins, peptides, STING agonists, small proteins and PNAs (peptide nucleic acids), some of which can initiate immune responses within tumors.

The first pHLIP® PET imaging agent (18F-NO2A-pHLIP), a pHLIP® fluorescent surgical imaging agent (ICG-pHLIP), and a pHLIP® therapeutic agent (pHLIP-PARPi, developed by Cybrexa Therapeutics) are entering clinical trials in 2019-2020.

YKR, OAA, and DME are founders of pHLIP, Inc. and have financial interests in the company.

Keywords: tumor acidity, TAMs, immune response, imaging and therapy.

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Wyatt LC, Lewis JS, Andreev OA, Reshetnyak YK, Engelman DM. Applications of pHLIP technology for cancer imaging and therapy. Trends Biotechnol, 2017, 35, 653-664.

## A171 / CD73 blockade potentiates radiation-induced tumor infiltration of conventional type I dendritic cells and abscopal anti-tumor effect in combination with immune checkpoint blockade

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Tumor-targeted radiation therapy (RT) can promote systemic and tumor-specific immune responses in cancer patients that are unresponsive to immune checkpoint blockade (ICB) [1]. RT-induced danger signals including release of nucleotides ATP and NAD by tumor cells contribute to tumor infiltration and activation of conventional type I dendritic cells (cDC1), which are uniquely capable of cross-presenting tumor antigens to CD8 T cells, and essential for anti-tumor immune responses [2-4]. In the tumor microenvironment (TME), hydrolysis of ATP and NAD, mediated by CD39 and CD38 respectively, generates precursors for CD73-mediated adenosine production that has broad immunosuppressive effects on both cDC1 and CD8 T cells [5]. In the present study, we tested the hypothesis that adenosine generation in irradiated tumors hinders RT-induced cDC1 infiltration and anti-tumor immune responses.

Tumor adenosine levels were assessed by liquid chromatography-mass spectrometry (LC-MS) in subcutaneous TSA mouse tumors and surface expression of CD73, CD38 and CD39 was measured by flow cytometry on murine (TSA and 4T1) and human (MDA-MB-231 and 4175TR) tumor cells. In order to assess the impact of CD73-mediated adenosine generation on the immunogenicity of 20Gy RT (previously shown ineffective at inducing cDC1 tumor infiltration [6]), Wild type (WT) or BATF3-deficient (BATF3-/-) mice (ablated development of cDC1) bearing subcutaneous TSA tumors were treated with 20Gy RT with or without concomitant CD73-blockade (anti-CD73 monoclonal antibody, TY/23) and monitored for tumor progression. Some mice were sacrificed 6 days after RT to analyze tumor-infiltrating DCs and T cells. cDC1s, generated from bone marrow cells cultured with FLT3L and GM-CSF, were CFSE-labeled and administered intravenously in mice bearing irradiated flank TSA tumors and contralateral non-irradiated tumors. Tumor infiltration of labeled cDC1s was assessed after 48 hours by flow cytometry. Systemic anti-tumor effect of CD73-blockade was assessed by combined treatment with RT and anti-CTLA-4 in the rapidly metastatic mammary carcinoma model 4T1. Lung metastatic burden was assessed on day 28 after tumor inoculation.

In TSA mouse tumors, a single dose of 20Gy RT significantly elevated adenosine levels after 24 hours. In vitro, CD73 and CD38 expression on murine and human breast cancer cells was increased following RT. CD73-blockade alone had no significant effect on tumor progression. However, in mice treated with 20Gy RT, CD73-blockade improved significantly RT-induced tumor control and mouse survival, an effect associated with enhanced tumor infiltration of activated cDC1 and CD8 T cells. Consistent with the hypothesis that cDC1 are essential for RT-induced anti-tumor responses, the observed synergy between RT and CD73 blockade was abrogated in BATF3-/- mice. Improved infiltration of cDC1 in irradiated tumors of anti-CD73-treated mice was confirmed in adoptive transfer experiments of ex vivo generated cDC1. Importantly, we observed that CD73-blockade in combination with ICB and RT induced systemic anti-tumor effects in the 4T1 model, reducing lung metastasis burden compared to RT, ICB or anti-CD73 alone.

Our findings show that RT upregulates expression of ectoenzymes in the non-canonical pathway of extracellular nucleotide catabolism promoting adenosine accumulation in irradiated tumors. We demonstrate that CD73 blockade can synergize with tumor-targeted RT to promote tumor infiltration of cDC1, primary tumor control and potentiate abscopal tumor effects in combination with ICB. With CD73 blocking antibodies currently in clinical testing, implementation of CD73 blockade with radiotherapy/immunotherapy combinations represents a promising treatment

option to improve responses in ICB refractory cancer patients.

Keywords: Adenosine, CD73, Radiation therapy, Dendritic cells.

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## A172 / Peptide modified siRA (E2F3) functionalized ZnO nanorods: A two-in-one approach for breast carcinoma

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Background: The delivery of combination therapeutic agents with different mechanism can cooperatively disallow progression and development of cancer. The present study demonstrates an innovative “two-in-one” approach based on systematic delivery of siRNA and doxorubicin (DOX) by poly ethylene glycol coated ZnOnanorods (ZnO NRs-PEG). The subsequent grafting of ZnOnanorods with PEG provides the space for loading both siRNA and DOX to form a “two-in-one” nano-complex, to specifically target tumor cells. In addition, utilizing a fresh siRNA transporting system combining double effects of folate targeting and bio-reducible disulfide bond linked siRNA-CPPs conjugate is developed to suppress E2F3 gene expression of breast cancer both in vitro and in vivo.

Methods: The bio synthesized ZnO NRs-PEG-siRNA-CPP-FA-DOX complex for its use as a breast cancer-targeted drug delivery system to down regulation of E2F3 expression, induced significant inhibitory effect and anticancer efficacy. To investigated the expression level of E2F3 signalling pathway by RT-PCR and Western blotting analysis and in vivotumor suppression study. Results: The antitumor activity of ZnO NRs-PEG-siRNA-CPP-FA-DOX were evaluating via in vitro and in vivo studies against breast cancer. We observed that ZnO NRs-PEG-siRNA-CPP-FA-DOX can efficiently deliver E2F3 siRNA into cancer cells, resulting in the down regulation of E2F3 expression, an induced significant inhibitory effect on the tumor growth. Further, results also confirmed that ZnO NRs-PEG-siRNA-CPP-FA-DOX nanorods had the strongest anti-tumor efficacy against MCF-7 tumor bearing xenograft mice.

Conclusion: In this study, we successfully developed a nanocarrier (ZnO NRs) for intravenous co-delivery of E2F3 siRNA and chemotherapeutic drug DOX. ZnO NRs-PEG-siRNA-CPPs-FA combining folate targeting, and CPPs was constructed to suppress E2F3 gene expression of breast cancer both in vitro and in vivo. Additionally, owing to the aforementioned advantages ZnO NRs-PEG-siRNA-CPP-FA-DOX remarkably inhibited tumor growth in a synergistic manner. Thus, a safe and efficient delivery system to target tumor growth by the co-delivery of siRNA and DOX will be much more attractive on the way to the clinic.

Keywords: ZnO Nanorods, DOX, siRNA, Codelivery, Brast cancer.

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## A173 / Group 3 Innate lymphoid cells are essential for Cisplatin mediated anti-tumor immune responses.

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Innate lymphoid cells (ILCs) are a native immune cell population discovered about ten years ago (1). ILCs are divided in subpopulations mirroring T helper subsets. ILC3s are the innate counterpart of CD4 Th17 cells, sharing their expression of the transcription factor RORgt and the production of IL-17 and IL-22. ILC3s are essential for the organogenesis of secondary lymphoid organs during embryogenesis and for intestinal homeostasis. In cancer, ILC3s have been shown to exert pro- or anti-tumor properties, depending on the microenvironment they are in (2, 3). However, we still do not know if ILCs and ILC3s in particular, can influence the efficacy of chemotherapies. Despite recent advances in immunotherapy and targeted therapies, cytotoxic agents remain a major weapon against cancer. In addition to their direct cytotoxic effect, chemotherapies can also affect the immune system in a positive or negative fashion. Cisplatin is a platinum salt based chemotherapy widely used in the treatment of various cancer types amongst which bladder, esophageal, breast and lung cancers. Although cisplatin is known to be a non-immunogenic drug, it still has immune-stimulating properties. A cisplatin treatment has been shown to induce a massive recruitment and proliferation of effector T cells as well as of antigen presenting cells expressing high levels of costimulatory molecules within the tumor bed in both human and mice (4). Our goal is to understand the role ILC3s may play in the anti-tumor immune responses triggered by a cisplatin treatment.

Using TC-1 tumor cells, a pulmonary cancer model, injected subcutaneously into WT mice, we studied by flow cytometry and in a time course fashion, the evolution of the immune populations found in the tumor after a cisplatin treatment. We were able to observe that ILC3s were increased in the tumor as early as one day after treatment when the other immune populations increase started at day three after treatment. In order to see if ILC3s were responsible for the latter increase in immune cells, we depleted them and could see that in absence of ILC3s, the immune infiltration of the tumor was greatly reduced. The absence of ILC3s also diminished the efficiency of cisplatin against tumor development. Here we show that ILC3s are a first mandatory step for the establishment of a potent immune response against the tumor after a cisplatin treatment. We hope that understanding the cellular and molecular mechanisms explaining cisplatin efficiency will help identify new potential targets to further improve its effects.

Keywords: Innate lymphoid cells, Chemotherapy, cancer, Cisplatin.

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## A174 / Synaptic actin remodeling underlies cancer cell resistance to cytotoxic lymphocytes

Hannah Wurzer (Luxembourg Institute of Health), Céline Hoffmann (Luxembourg Institute of Health), Jerome Mastio (Luxembourg Institute of Health), Clement Thomas (Luxembourg Institute of Health).

Immune evasion is a major hallmark of cancer. Significant progress has been made in investigating how tumors evade recognition and destruction by the immune system. However, approaches on antagonizing these escape strategies are often overcome in a short time. Using live cell imaging confocal microscopy and imaging flow cytometry we established that rapid actin cytoskeleton remodeling in cancer cells can promote their survival during cytotoxic lymphocyte attack. Tumor cells that respond to lymphocyte contact with a quick and massive accumulation of F-actin at the region of the immunological synapse, a process we termed “actin response”, survive cytotoxicity assays and can detach from the immune cell without undergoing apoptosis. From a mechanistic standpoint, the rapid accumulation of polymerized Factin at the immunological synapse is accompanied by reduced levels of cytotoxic molecules inside target cancer cells. Our mechanistic investigations suggest that the actin response is at the heart of several immune evasion strategies. We report similar findings in different types of cancer as well as murine cancer cell models, indicating a conservation of this escape strategy. In vivo data show that stimulating the actin response by interfering with specific mediators of actin polymerization dramatically increases tumor growth in immune competent mice. Together our work identifies the actin cytoskeleton as a potentially vulnerable property of cancer cells, with the possibility to target specific actin filament populations or actin-binding proteins that are selectively upregulated or show altered functions in cancers.

Keywords: Cytoskeleton, Cytotoxic lymphocytes.

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## A175 / The effect of inducing mismatch repair deficiency on low tumor mutation burden tumors and anti-tumor immunity

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Immune checkpoint inhibitors (ICIs) have improved patient survival across a number of cancers, especially in high tumor mutation burden (TMB) tumors. However, most cancer patients, across the spectrum of malignancies, still do not respond to ICIs. Patients with DNA mismatch repair (MMR)-deficient tumors show high response rates to ICIs, regardless of the type of cancer (1). This has led to the first FDA approval of pembrolizumab (anti-PD1) for any type malignancies with MMR deficiency. Although this phenomenon is attributed to higher levels in neoantigens in MMR-deficient tumors the underlying immune mechanisms behind these heightened responses are not understood (2). It is also not clear whether tumors with low TMB such as neuroblastoma could be rendered responsive to ICIs by inducing MMR deficiency in them. Here we used CRISPR/CAS9 genome editing to knock out (KO) MLH1, encoding a crucial molecule in the MMR pathway, in mouse neuroblastoma (Neuro-2a) cells – refractory to ICIs – to induce MMR deficiency. To analyze tumor growth inhibition in response to ICIs and T-cell immune profile, tumors with intact or induced MMR deficiency were injected subcutaneously into immunocompetent mice. Tumor growth and surrogate measures of animal survival were measured after treatment with anti-PD1 antibodies. In a separate set of experiments, mice were euthanized after tumors reached ~1400 mm3; spleen, lymph nodes, and tumor-infiltrating lymphocytes (TILs) were harvested and analyzed for activation, exhaustion, and effector markers, allowing for in-depth flow cytometric analysis of T-cell subsets in these mice. We also examined MHC-I and PD-L1 levels on tumors upon induction of MMR deficiency. Our data demonstrate that mice injected with tumors with induced MMR-deficiency exhibit a robust immune response following anti-PD1 therapy, including rejection of a subset of tumors. We observed increased total T-cell populations in these mice and changes in some immune markers. Furthermore, tumor cells increased their PD-L1 levels and decreased their MHC-I levels over time following induction of MMR deficiency, in an immunoediting-independent manner, as a potential mechanism of resistance to anti-PD1 therapy in response to induction of MMR deficiency.

In conclusion, our data show that inducing MMR deficiency sensitizes tumors with naturally low TMB to ICIs while inducing phenotypic changes on immune and tumor cells.

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Keywords: mismatch repair pathway, DNA damage, neoantigen, checkpoint inhibitors.

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## A176 / Helicobacter pylori accelerates metaplasia and dysplasia in a mouse model of gastric cancer pre-neoplastic progression

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Gastric cancer is the fifth most common cancer and third-leading cause of cancer deaths worldwide (1). Almost 90% of gastric cancer is attributable to stomach infection with Helicobacter pylori (Hp) (2), a bacterium that infects half of humans (3). Hp is now known to initiate an inflammatory cascade that can result in gastric cancer: infection causes chronic gastritis, which can induce loss of gastric acid-producing parietal cells. In response, digestive enzyme-producing chief cells can transdifferentiate, resulting in an inflammatory state called spasmolytic polypeptide-expressing metaplasia (SPEM), which can progress to intestinal metaplasia (IM), dysplasia, and finally to gastric cancer (4-10). However, most infected people do not develop cancer, and the specific mechanism(s) through which Hp infection leads to cancer – beyond initiating inflammation – are not fully understood. Here we used a transgenic mouse model in which the induction of an oncogenic Kras allele in the chief cells of the stomach rapidly causes SPEM and IM in the absence of infection (11). We examined the effects of concomitant Hp infection and Kras induction, compared to either Hp infection alone or Kras induction alone. Our preliminary results show that Hp infection coupled with oncogenic Kras expression leads to: i) severe histopathologic alterations marked by increased epithelial thickness, oxyntic atrophy (loss of chief and parietal cells), and accelerated metaplasia progression; ii) a significant increase in dysplastic glands throughout the stomach body; and iii) enhanced inflammation marked by infiltration of macrophages and CD4+ and CD8+ T cell subsets. Despite this striking immunopathology, Hp is able to persistently colonize the stomach during the onset of these pre-cancerous changes, and to maintain the function of its type IV secretion system, a bacterial virulence factor implicated in gastric cancer development. Collectively, these studies point to a previously unappreciated role for Hp in driving gastric carcinogenesis beyond the initiation of inflammation, through inflammatory and structural modifications to the gastric mucosa. These studies may lead to new advances in gastric cancer diagnosis and treatment strategies, which are urgently needed.

Keywords: gastric cancer, bacteria, metaplasia, dysplasia.

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## A177 / B cells sustain inflammation and predict response to immune checkpoint blockade in human melanoma

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Tumor associated inflammation predicts response to immune checkpoint blockade in human melanoma. Established mechanisms of therapy response and resistance center on the contribution of anti-tumor T cell responses. Here we show that tumor-associated B cells are vital to tumor associated inflammation.

In human melanoma, tumor-associated B cells are primarily located at the invasive tumor-stroma margin arguing for a preferentially cell contact-independent communication with tumor cells. We therefore exposed in vitro peripheral blood- and melanoma-derived B cells to the secretome from autologous melanoma cells and observed induction of several pro- as well as anti-inflammatory factors. Exposed B cells differentiated towards a plasmablast-like phenotype with marked down-regulation of surface CD20 and upregulation of CD38 expression. In vivo, this functional B cell phenotype could be reconciled as a distinct B cell cluster in public single-cell RNA-seq data as well as by 7 color multiplex immunostaining from human melanoma samples. RNA-seq and multiplex immunostaining data also revealed that anti-CD20 immunotherapy not only significantly reduced the number of tertiary lymphoid follicles and depleted plasmablast-like TAB from human tumors, but also led to a pronounced decrease in tumor inflammation signatures as well as CD8+ T cell numbers. Single-cell RNA-seq data demonstrated the expression of T cell chemoattractants CCL5, CCL4, and CCL28 in plasmablast-like TAB further supporting their critical role in recruitment of immune cells and sustaining tumor inflammation.

The potential clinical implications of our observations are demonstrated in large-scale whole tissue and single-cell RNA-seq datasets. Here, the plasmablast-like TAB cell signature correlated with T cell abundance, inflammation and improved patient survival in the TCGA cutaneous melanoma cohort. Most strikingly, the frequency of plasmablast-like TAB in pretherapy melanoma samples was predictive of response to immune checkpoint blockade therapy in the Riaz et al. (Cell 2017) and Sade-Feldmann et al. (Cell 2018) melanoma cohorts. Consistently, in a surrogate assay of T cell activation, melanoma secretomes induced autologous B cells to significantly increase the activation of PD-1-expressing Jurkat T cells by PD-1 blockade.

Together, our data argue that tumor-associated B cells orchestrate and sustain tumor inflammation, recruit CD8+ T effector cells and may represent a predictor for response and survival to immune checkpoint blockade in human melanoma.

Keywords: tumor associated B cells, melanoma, inflammation, tumor microenvironment.

## A178 / FcγRIIb engagement is required for αOX40 antibody-mediated activation of human tumor-infiltrating T cells

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OX40 (CD134) is a co-stimulatory member of the TNFR family that is transiently expressed on T cells upon antigen recognition. Thus, OX40 targeting emerged as a potential immunotherapeutic treatment avenue to reinvigorate anti-tumor activities of tumor-infiltrating lymphocytes (TILs) by enhancing effector T cell expansion and suppressing Treg activity.

The aim of this study was to investigate mechanisms and requirements for effective OX40 co-stimulation of primary human TILs. Therefore, we isolated TILs, leukocytes from surrounding tumor-free tissues as well as peripheral blood mononuclear cells (PBMC) of hepatocellular carcinoma (HCC; n=25) and colorectal cancer (CRC; n=46) patients that underwent surgical resection. OX40 expression was determined by flow cytometry immediately after cell isolation. To assess effects of OX40 co-stimulation on PBMC and TILs, cells were cultured for up to 8 days in vitro in the presence of CD3/CD28 activation beads with or without hexameric OX40 Ligand or agonistic human αOX40 antibodies. Subsequently, T cell numbers as well as secreted cytokine levels were determined by flow cytometry and multiplex cytokine assays respectively.

First, we assessed OX40 expression on T cells from tumor, tumor-free tissue and peripheral blood. Tumor-derived T cells displayed higher OX40 levels as compared to T cells from blood or surrounding tumor-free tissue. Within TILs of both cancer types, activated CD4+Foxp3hiCD45RA- Treg (aTreg) and activated CD4+Foxp3dimCD45RA- Th (aTh) cells showed higher OX40 levels as compared to CD4+ Foxp3- Th and CD8+ TILs. Addition of hexameric OX40 Ligand to TILs cultured in the presence of CD3/CD28 activation beads enhanced expansion of CD4+ and CD8+ TILs and IFN-γ secretion in a dose-dependent manner. While soluble human agonistic αOX40 antibody failed to enhance TIL expansion, αOX40 antibody multimerized on beads significantly stimulated TIL proliferation. This suggested that clustering of OX40 molecules is a prerequisite for efficient signal transduction. To determine whether αOX40-mediated co-stimulation requires FcγRIIb engagement, TILs were treated with an Fc-engineered human αOX40 IgG1 antibody with selectively enhanced FcγRIIb affinity, termed αOX40_v12. Soluble αOX40_v12 efficiently enhanced CD4+ as well as CD8+ TIL expansion while antibody-mediated blockade of FcγRIIb abrogated this effect. Stimulation with αOX40_v12 enhanced secretion of IFN-γ as well as Th2/9 cytokines whereas IL-17 remained unchanged. FcγRIIb was detected on most CD45+ CD3- TIL subsets including B cells, monocytes, cDC and to a smaller extent NK cells, whereas T cells did not express FcγRIIb. Accordingly, αOX40_v12 failed to enhance expansion of CD3-purified TIL, demonstrating that the presence of FcγRIIb on non-T cells is critical for OX40 co-stimulation. To understand whether OX40-mediated enhanced TIL proliferation in our assays was based on reduced Treg suppression or a direct activation of effector T cells, we depleted CD25hi TILs by magnetic cell separation. In the absence of Tregs, αOX40_v12 stimulation still enhanced CD4+ and CD8+ TIL expansion, thus indicating that a direct activation of effector T cells at least partially contributes to the enhanced TIL proliferation.

In conclusion, we showed for the first time that OX40 targeting has the potential to reinvigorate expansion and activity of tumor-derived human T cells. Additionally, our data indicate that effective co-stimulation of human T cells by OX40 targeting requires multimerization of OX40 molecules which can be achieved by FcγRIIb-mediated antibody crosslinking as shown previously for other members of the TNFR family including CD40. Thus, by modulating the affinity to FcγRIIb, Fc-engineering is a critical tool to harness the full potential of αOX40 cancer immunotherapy.

Keywords: OX40 immunotherapy, liver and colorectal cancer, FcγRIIb-mediated antibody crosslinking, Fc engineering.

## A179 / CCR8 as a novel biomarker for the specific targeting of a highly suppressive tumor infiltrating regulatory T cell subset

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Aside from cancer cells, the tumor microenvironment (TME) contains a vast array of immune cells which are known to contribute significantly to tumor growth and dissemination. These immune cells are therefore considered interesting targets for cancer therapy. Strikingly, detailed studies regarding the role of chemokines and chemokine receptor expression on tumor-resident immune cells are largely lacking.

Through the use of single cell RNA sequencing we unraveled the T cell complexity in Lewis Lung carcinoma and were able to identify two distinct regulatory T cell (TI-Treg) subsets within the TME. The Chemokine (C-C motif) receptor 8 (CCR8) appeared specifically expressed by the highly activated TI-Treg subset. These findings were confirmed at the protein and functional level, where the CCR8+ TI-Tregs showed higher expression of a plethora of activation markers and immune checkpoint molecules, including LAG-3 and OX40, and showed a superior suppressive capacity. Interestingly, this upregulation of CCR8 on the activated Tregs was specific for the TME and appeared to be antigen-induced. Moreover, these findings also translate to human cancer where we found that CCR8 upregulation also appeared to be specific for the TI-Tregs. These results indicate that CCR8 can be used as a biomarker for this pro-tumoral TI-Treg subset. However, comParison of WT TI-Tregs to CCR8-KO TI-Tregs showed that a loss of CCR8 expression did not influence TI-Treg recruitment to the TME, nor their activation status or their suppressive capacity, indicating that CCR8 does not play an important functional role on TI-Tregs. Aside from CCR8, single cell RNA sequencing of the whole TME showed that the CCR8-specific ligand, CCL8, was specifically and highly expressed by pro-tumoral M2-like TAMs. However, the exact role of CCL8 remains elusive.

Overall, the unique expression-profile of CCR8 allows it to be used as a potent biomarker for the therapeutic targeting of the pro-tumoral LAG-3High OX40High TI-Treg subset. This highly specific targeting would allow us to prevent systemic Treg depletion in cancer patients and avoid the induction of autoimmune complications.

Keywords : Immuno-oncology, CCR8, Regulatory T cells, Immunotherapy.

## A180 / Immunomodulatory effect of Hepatocyte Growth Factor on monocytes in human gastric cancer

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Understanding the mechanisms involved in tumor-induced immunosuppression is currently of major interest. Among these immunosuppressive pathways, induction of regulatory T cells (Treg) can inhibit the development of an efficient anti-tumor immune response. At physiological state, Treg induction is partly dependent of a subtype of dendritic cells (DC) characterized by an immature phenotype. [1] Hepatocyte Growth Factor (HGF) and its receptor c-met are involved in numerous cellular functions such as proliferation, migration and survival. Deregulation of the HGF/c-Met signalling occurs in several cancers such as gastric adenocarcinoma (GA), leading to aggressive cellular invasiveness [2] [3]. Yet, it has been shown in vitro in healthy volunteers (HV) that the HGF/c-Met pathway could promote the development of Treg via DC [4], but this role has never been reported in human malignancies. We studied the immunomodulatory effects of HGF in GA patients.

Peripheral blood from GA patients was collected and cMet expression on mononuclear cells was analysed by flow cytometry. Monocytes were isolated by magnetic separation and cultured with GM-CSF and IL-4 (standard condition to induce DCs) with or without HGF for 6 days. In some experiments at day 6, cells were matured by LPS for 24 hours. At day 7, cell phenotype and cytokine production were assessed respectively by flow cytometry and ELISA in culture supernatants. Then, monocytes previously differenciated in presence or absence of HGF were co-cultured with allogenic T CD4+ lymphocytes (LT), isolated from peripheral mononuclear cells of HV by magnetic separation. After 7 days of co-culture, LT phenotype was analyzed by flow cytometry.

47 patients with a GA were included (median age: 64.4 years; male = 76%). No expression of c-Met could be detected on the surface of LT or Treg (0.36+/-0.13 % and 0.55+/-0.20 % respectively), suggesting that HGF could not act on lymphocytes in a direct manner. However, monocytes, that are precursor cells of DC, expressed c-Met (15.95+/-2.97%). C-Met expression was the same in the three monocytes subtypes (classical, intermediary and non classical). There was no difference of c-Met expression according to the histological type of adenocarcinoma (intestinal type, diffuse or mixed type), or to the tumor location (gastric body or oesogastric junction). However, c-Met expression on monocytes was higher in patients with a tumor burden (localized or metastatic) than in patients with no tumor burden (patients who underwent gastrectomy). Furthermore, we analysed cMet expression in one patient before and after tumor resection by gastrectomy. We observed that cMet expression on peripheral monocytes decreased after the tumor resection. Treatment of monocytes with HGF in the presence of GM-CSF and IL-4 induces the development of cells expressing the DC markers CD11c and HLA-DR. Conditioning of these cells with HGF decreased the expression of CD80, CD83 and CD86 co-stimulatory molecules and inhibited their maturation induced by LPS. Furthermore, HGF stimulation increased the secretion of the protolerogenic cytokine IL-10. [5] Lastly, when co-cultured with monocytes previously treated with HGF, expression of Treg markers by LT (high expression of CD25, high expression of Foxp3 and low expression of CD127) was significantly increased, compared to the control condition.

In conclusion, peripheral monocytes from GA patients express the HGF receptor c-Met. In the presence of HGF, monocytes differentiated into cells with DC features and a protolerogenic phenotype. In addition, monocytes previously differentiated in presence of HGF could induce the expression of Treg markers by allogenic LT co-cultured in vitro. Thus, HGF could be a novel immune escape mechanism in cancer and could be targeted not only for its direct anti-tumor effect but also for its immunomodulatory properties.

Keywords : Regulatory T lymphocytes, Hepatocyte Growth Factor, Monocytes, Dendritic cells.

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## A181 / Proteases activated during lethal heat shock participate in antigen processing and influence the immunogenicity of tumor cell death

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Immunogenic cell death (ICD) is a form of apoptosis that facilitates the induction of anti-tumor immune responses. ICD in tumor cells is a crucial determinant of therapeutic efficacy in cancer and relies on both the release of tumor antigens and immune-stimulatory molecules called damage-associated molecular patterns (DAMPs)[1]. While the programmed, sequential release of DAMPs during ICD is well characterized, the fate of antigen in dying tumor cells is poorly defined[1], [2]. However, in other contexts, the activation of particular cell stress and death pathways in antigen donor cells has been shown to influence subsequent antigen cross-presentation[3], 4. We hypothesized that similar mechanisms may operate in tumor cells undergoing ICD. Here we investigated whether proteasome-independent proteases, activated during lethal heat shock, participate in antigen processing and influence the immunogenicity of tumor cell death.

Using the MC38 murine colorectal cancer model, tumor cell death was induced by lethal heat-treatment (56ºC for 90 minutes) and validated as bona fide ICD by vaccination followed by live tumor challenge5. A self-quenched, fluorogenic ovalbumin conjugate (DQ-OVA), which generates dye-labeled peptides upon proteolytic degradation, was utilised to assess proteolytic activity, antigen processing and peptide localization in heat-killed (HK) tumor cells. Marked protease activity was observed in HK tumor cells, which were capable of processing antigen and binding resultant peptides. Spinning-disk confocal microscopy and 3D reconstruction revealed antigenic peptides processed by tumor-derived proteases associated with the inner leaflet of the plasma membrane, accumulating at high concentrations. Interestingly, HK tumor cells failed to induce CD4+ T-cell activation, but effectively induced cross-presentation to elicit CD8+ T-cell activation in vitro. Prolonging antigen exposure to protease activity in HK tumor cells also increased subsequent cross-presentation in a time-dependent manner. CD8+ T-cells expanded using HK tumor cells produced IFNγ, exhibited tumor cytotoxicity in vitro and provided protective antitumor immunity when adoptively transferred into tumor-bearing mice. Selective inhibition of tumor-derived calpains markedly reduced the cross-presentation of antigen associated with HK tumor cells in vitro, while selective inhibition of cathepsins L and B, as well as pan-caspase activity, resulted in a partial reduction. Combination of selective calpain, cathepsin and caspase inhibition had an additive effect, while broad tumor-derived protease inhibition almost entirely abrogated cross-presentation. Importantly, these observations were attributable to the inhibition of tumor-derived-proteases and not those present in antigen-presenting cells.

Together, these results indicate that tumor-derived proteases, activated during lethal heat shock, participate in antigenic processing that subsequently enhances cross-presentation and the immunogenic potential of tumor cell death. Furthermore, our findings suggest a dominant role for calpains in this process. Promoting calpain activity in dying tumor cells may therefore represent a novel approach to improve the efficacy of immune-based cancer therapies.

Keywords: Immunogenic cell death, Heat shock, Proteases, Tumor lysates.

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## A182 / The diverse immunopeptidome of melanoma

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Antigen-recognition by CD8+ T cells is governed largely by the pool of peptide antigens presented on the cell surface in the context of HLA class I complexes. Recent studies have shown not only a high degree of plasticity in the immunopeptidome, but also that a considerable fraction of all presented peptides are generated through proteasome-mediated splicing of non-contiguous regions of proteins to form novel peptide antigens. Here we used high-resolution mass-spectrometry combined with new bioinformatic approaches to characterize the immunopeptidome of melanoma cells in the presence or absence of interferon-gamma. In total, we identified 29, 934 peptides and demonstrate that interferon-gamma induces marked changes in the peptidome (with an overlap of only 49.8% between conditions as revealed by data independent acquisition mass spectrometry). Around 6% (1, 774) of the peptides were identified as cis-spliced peptides, and 712 peptides (674 linear, 38 spliced peptides) were derived from known melanoma-associated antigens. Of note, several peptides showed in vitro immunogenicity across multiple melanoma patients. These observations highlight the breadth and complexity of the repertoire of immunogenic peptides that may be exploited therapeutically and suggest that spliced peptides may be a major class of tumour antigens.

Keywords: Immunopeptidome, Cis-splicing, Melanoma, Antigen processing.

## A183 / An optimized in vitro model to uncover the transcriptomic landscape of activated tumor-reactive T cells

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Cytotoxic T-lymphocyte (CTL) recognition of tumor-antigens, followed by activation of killing functions, represents the final common pathway of immune-mediated tumor regression. However,

the complete functional repertoire of tumor-reactive CTLs is still largely unclear. To characterize the precise molecular events leading to CTL activation and killing, we combined various cellular and molecular techniques to accurately reproduce the CTL-tumor cell interactions occurring within the tumor microenvironment (TME) of individual cancer patients. Our aim was to generate a robust workflow, allowing the isolation and transcriptomic characterization of tumor-reactive CTLs upon recognition of autologous tumor cells in vitro. We isolated tumor-reactive CTLs from the TME of 12 patients of varying histologies (metastatic melanoma, sarcoma, ovarian cancer), and following recognition of naturally presented tumor-antigens in vitro, tumor-reactive CTLs were purified via magnetic-activated cell sorting and their mRNA sequenced. Our analysis demonstrated that CTLs recognizing autologous tumor antigens consistently upregulated many expected genes associated with activation and cytotoxicity programs (e.g. TNFRSF9, IFNG, GZMB, TNF, HAVCR2), thereby confirming the viability of our method. In addition, numerous additional genes (data not shown) associated with presently uncharacterized functions were found to be highly upregulated, indicating additional roles for CTLs in the TME and further eluding to the potential of this method. Our results show that this workflow is a tool that can feasibly be used with patient-derived material to directly study natural tumor-CTL interactions and the functional repertoire of CTLs.

Keywords: cytotoxic T cell, transcriptomic analysis, tumor-reactive T cell.

## A184 / Salt-inducible kinase 3 facilitates tumor cell resistance against cytotoxic T cell attack by shifting TNF signaling from apoptosis to survival

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Immune checkpoint blockade (ICB) is a breakthrough cancer therapy resulting in - for the first time - long-lasting patient survival. ICB functions by reactivating the patient's immune system (e.g. cytotoxic T cells) against tumor cells leading to disease remission. However, the majority of patients do not benefit from current immunotherapy treatments because tumors employ an arsenal of checkpoint and resistance pathways to evade immune attack. Our group uses the iOTarg RNAi screening platform to discover targets and pathways that induce immune resistance. Here we report that desensitization to immune cell-derived TNF is a key immune evasion strategy employed by tumor cells, which is tightly regulated and orchestrated by the tumor-intrinsic activity of salt-inducible kinase 3 (SIK3). Inhibition of SIK3 using various independent genetic tools - including siRNAs, shRNA, CRISPR – induced a strong sensitization of various human and murine (MC38) tumor cell lines to T cell or TNF-mediated apoptosis. Mechanistically, SIK3 induced the phosphorylation of histone deacetylase HDAC4, upon TNFR1 triggering, preventing its nuclear shuttling. Excluding HDAC4 from the nucleus leads to reduced promotor region deacetylation around NF-κB binding sites, increased chromatin accessibility and subsequently enhanced expression of NF-κB driven pro-survival genes, as observed via ATAC-seq analysis. To understand the clinical relevance of the above biology in patient cohorts, we generated a SIK3-dependent gene signature of TNF-mediated NF-κB activation in a cell line model. By overlaying this gene signature onto the TCGA pancreatic cancer cohort of 185 patients, we identified a large majority of patients with an upregulated SIK3 pathway signature which correlated with poor prognosis despite increased cytotoxic T cell (CTL) activity score, emphasizing the role of SIK3 as a key regulator of tumor cell resistance against immune attack. We are investigating SIK3 as a target for modulation by small molecule inhibitors in order to sensitize tumor cells to immune attack. As a proof-of-concept, we used known SIK3 inhibitors HG-9-91-01 and dasatinib, as well as a synthesized SIK3-inactive dasatinib derivative (OMX-0326), for further studies. Despite the broad kinase inhibition profile of the above inhibitors, only compounds with the ability to inhibit SIK3 sensitized a large set of human tumor cell lines, as well as MC38 murine tumor cell line, towards TNF-induced apoptosis via a dose-dependent reduction of NF-κB's transcriptional activity. Hence, the anti-tumor activity of SIK3 inhibitors is based on abrogation of the TNF/SIK3 driven NF-κB pathway. Furthermore, MC38 tumor-bearing mice treated with SIK3 inhibitor demonstrated a significant tumor growth inhibition in vivo and, in combination with anti-PD1 treatment, showed significant increase in CTL activation and CTL to Treg ratio. In summary, we report a novel role for tumor-intrinsic SIK3 as a regulator of resistance to immune cell attack via modulation of the NF-kB pathway. Inhibition of SIK3 via small molecules sensitizes tumor cells to immune attack in vitro and in vivo, making it a promising drug target for cancer treatment. Our work further allows for selection of the most beneficial patient population for treatment with novel proprietary SIK3 inhibitors.

Keywords: Immune Checkpointt, High-throughput screening, Resistance to immune attack, TNF signaling.

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## A185 / Targeting citrullinated vimentin and enolase with cytotoxic CD4 T cells, relies upon MHC-II expression by tumors, reduces myeloid suppressor cells and directly kills tumor cells.

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Post-translational modifications in cancer cells result in changes to proteins which cause them to be recognised by the immune system. One such modification is citrullination, the conversion of arginine residues to citrulline by peptidylarginine deiminase (PAD) enzymes. In this study we show in both HLA-DR4 and HLA-DP4 transgenic mice that a combination of two citrullinated vimentin peptides and one enolase peptide (Modi-1) show similar Th1 responses compared to single immunisations. Modi-1 induced potent anti-tumor responses in HLA-DR4 or HLA-DP4 transgenic mice against both B16 melanoma and ID8 ovarian tumors expressing either constitutive or IFNγ inducible DR4 or DP4. In contrast, there was no anti-tumour response from wild type peptides or adjuvant alone. CD4 depletion but not CD8 depletion abrogated the primary anti-tumor response as well as recall response to tumor rechallenge. The role of CD4 T cells was further validated as successful tumor regression was consistently associated with an increase in CD4 infiltrate in the tumor and a reduction in tumor associated myeloid suppressor cells Indeed, direct CD4 killing was crucial as only tumors expressing class II were rejected. A comParison of different TLR stimulating adjuvants showed that Modi-1 induced strong Th1 responses when combined with GMCSF, TLR9/TLR4, TLR9, TLR3, TLR2 and TLR7 agonists. Linking the TLR2 agonists to the peptides allowed the vaccine dose to be reduced 10-100 fold without loss of anti-tumor activity. These studies suggest that low dose of citrullinated peptides linked to TLR2 ligand should be rapidly translated to the clinic for the treatment of cancer. Furthermore, ovarian cancer patients show a CD4 proliferative response to one or more of the citrullinated peptides

which is similar to that in healthy donors. These results suggest that Modi-1 could be an effective vaccine for ovarian cancer patients.

Keywords : Citrullination, CD4 T cell, vaccination.

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## A186 / Resolving immune suppressive signatures in the tumor-microenvironment of pancreas adenocarcinoma

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Pancreatic adenocarcinoma (PDAC) is one of the deadliest cancers, with a 5-year survival rate of 8%. Due to PDAC's highly aggressive nature and early invasion to the surrounding vasculature, the majority of tumors are inoperable. Additionally, other treatment strategies such as chemo-, targeted- or immunotherapy remain ineffective in curing patients.

Treatment failure has been attributed to the complex PDAC tumor microenvironment. The PDAC tumor microenvironment is characterized by abundant, reactive, desmoplastic stroma, resulting in a nutrient poor immunosuppressive environment. The majority of immune infiltrates are cells of the myeloid lineage, with myeloid derived suppressor cells (MDSCs) as well as tumor associated macrophages (TAMs) contributing to local immune suppression. Cancer associated fibroblasts (CAFs) and extracellular matrix proteins also contribute to the aggressive behavior of the tumor by promoting proliferation, progression, invasion, metastasis and chemo resistance.

It is well established that tumor cells have altered glycan expression in glycoproteins and glycolipid, referred to as the tumor glyco-code, that influence tumor growth and metastasis. In recent years, there has been an increasing interest in the interplay between the tumor glyco-code and the immune system, since many immune cells can recognize glycans through their immune inhibitory lectin receptors. For example, we have shown that sialylation in B16 melanoma tolerizes NK cells and promotes Treg formation favoring tumor growth.

We here show using transcriptomic data, that PDAC biopsies have enhanced sialylation. The presence of sialic acids in human PDAC tumors was confirmed by tissue analysis using plant-lectin stainings. Sialic acids are recognized by a family of immune receptors called SIGLEC's, of which most contain an ITIM motif causing immune suppression upon binding of sialic acids. Interestingly, only SIGLEC-7 and SIGLEC-9 ligands were present on PDAC tumor cell lines. The receptors SIGLEC-7 and SIGLEC-9 are expressed on circulating monocytes, and were found in human PDAC biopsies, suggesting that sialic acids on tumor cells can interact with these monocytes and modify monocyte function. Indeed, we show that PDAC tumor cells induced specifically tumor-associated macrophage (TAM) differentiation from monocytes via SIGLEC-7 and SIGLEC-9 in vitro. Additionally, the presence of SIGLEC ligands in PDAC patients correlated with a TAM signature in the tumor.

In conclusion, PDAC tumor cells upregulate sialic acids by which they induce TAM differentiation from monocytes via SIGLEC-7 and SIGLEC-9, thereby contributing to immune suppressive signatures in PDAC. Future therapy removing sialic acids, needs to reveal the potency of glyco-immune interference for optimal cancer immunotherapy.

Keywords : Pancreatic cancer, Glycosylation, Sialic acid, Tumor-associated macrophage.

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## A187 / Deciphering the tumor-natural killer cell interactions to develop immunotherapies for lung adenocarcinoma

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Cancer immunotherapy, which bolsters patient's immune system to attack tumor cells, results in durable responses in several cancer types (1-4). Inhibitory antibodies against immune checkpoint proteins, CTLA-4 and PD-1, induce T cell mediated anti-tumor immunity resulting in objective response rates over 20% and durable disease responses over 10 years (5). Despite these favorable outcomes, a considerable subset of patients does not respond to such therapies. In these patients, reinvigoration of T cells may not be sufficient to eradicate tumors and that activation of additional immune cells may be required.

Natural killer (NK) cells are effector lymphocytes of the innate immune system and participate in early control of virus infection and tumor immunosurveillance both in humans and mice (6-8). In mouse, lack of major histocompatibility complex (MHC) class I expression (9) or presence of NKG2D receptor ligands (10, 11) renders tumor cells susceptible to NK cell mediated cytotoxicity and results in rejection of transplanted tumors. Activating receptors on NK cells, such as NKG2D that recognizes stress-induced ligand expression on tumor cells (12) can bolster NK cell cytotoxicity against target cells. In addition, engagement of the activating receptor CD16 (FcγRIIIa) on NK cells potently mediates antibody-dependent cellular cytotoxicity (ADCC) induced by therapeutic tumor targeting monoclonal antibodies such as trastuzumab (anti-HER2), cetuximab (anti-EGFR) or rituximab (anti-CD20) (13). An alternative therapeutic strategy is the application of multi-specific antibodies that co-engage tumor antigens and NK cell receptors. In addition to conserving the ADCC effects, these multi-specific antibodies can bridge the NK cells to tumor cells. This strategy is beginning to yielded promising results in patients with Hodgkin's lymphoma (14).

Although our understanding of tumor immunosurveillance and immunotherapies were greatly enhanced by tumor transplant mouse models, a major limitation of these models was that tumors grew outside of their native tissue and could not faithfully recapitulate the complexity of the physiologic tumor microenvironment. To try to better understand the dynamic interactions between tumor and immune cells in tumors that arise in their native tissue, we utilize the autochthonous KrasLSL-G12D/+; P53flox/flox (KP) mouse model, which enable KRASG12D expression and P53 deletion upon intratracheal delivery of lentiviral Cre recombinase leading to the formation of lung adenocarcinoma, that recapitulates the genetic and histopathological features of the human disease (15, 16).

In this study, we examined the abundance and activity of NK cells in KP mouse model during lung cancer initiation and progression. Flow cytometric analysis of NK1.1+CD3-CD49a-CD49b+Eomes+ cells 10 weeks post-tumor initiation revealed a significant increase in mature NK cells with effector function in early stages of the tumor development. These conventional NK cells displayed enhanced activity in the tumor-bearing lungs evidenced by increased IFNγ and Granzyme B production. Independently, we observed significantly increased numbers of NKp46+ cells in tumor-bearing lungs using immunohistochemical analysis. As the tumors progressed, the number of NK cells returned to normal levels observed in control lungs. Decreased cytokine secretion and degranulation capabilities suggested that NK cells became less functional at later stages of the tumor development. As a therapeutic strategy to reinvigorate the NK cell function and to promote anti-tumor immunity, we are testing the in vivo efficacy of NK cell engagers, simultaneously targeting activating NK cell receptors and tumor cell surface antigens.

Keywords : NK cells, Innate immunity, Immunotherapies, Bispecific antibodies.

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## A188 / Loss of EMC inhibits tumor growth through enhanced adaptive immune response

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Checkpoint inhibitors have shown remarkable responses in patients with cancer. However, only a small percentage of patients with immunogenic tumors responded well, suggesting necessity of additional treatments, especially ones that can turn the non-immunogenic tumors immunogenic. We performed a genome-wide CRISPR-cas9 screening in mouse cancer cell lines to identify mechanism(s) regulating MHC class I cell surface expression, where down-regulation links to resistant to immune surveillance. Guide RNAs for most of core subunits of ER membrane protein complex (EMC) were recovered from the screen, along with genes regulating interferon-gamma signaling and nonsense-mediated decay. Deletion of each EMC subunits, especially EMC1 or EMC3, increased MHC class I level at the cell surface, which was further augmented by interferon-gamma treatment. EMC1- or EMC3-deleted cancer cells implanted in syngeneic mice developed tumors initially, which then resolved completely by itself. In contrast, EMC-deficient cells implanted in Rag1-deficient mice showed no growth disadvantage, indicating that growth inhibition of EMC-deleted cancer cells in syngeneic mice was mediated by adaptive immune responses. In addition, we observed increased CD45+ leukocytes infiltration into EMC1-deficient tumors and enhanced CD8 T-cells proliferation when co-cultured with EMC1-deleted tumor cells. EMC has been shown to be involved in proper protein folding and induces ER stress when deleted in yeast. Induced ER stress by thapsigargin in EMC-deficient cells did not upregulated MHC class I, indicating the EMC acts on MHC class I through different mechanisms. Interestingly, deletion of EMC increased expression of genes regulating antigen presentation, including TAP1, TAP2, beta2M, as well as subunits of immunoproteasome, whereas PD-L1 level was not affected. This study suggests that targeting EMC in tumor cells might represent a novel therapeutic strategy worth further investigation.

Keywords: ER membrane protein complex (EMC), MHC class I.

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## A189 / Analysis of the glyco-code in pancreatic cancer identifies novel immune regulatory circuits

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There is a clear need for new diagnostic and therapeutic approaches in pancreatic ductal adenocarcinoma (PDAC), that remains as a lethal disease with a 5-year survival rate of only 7%. Immunotherapies, highly effective in other types of cancer, fail to succeed in PDAC, probably due to its low immunogenicity. A key hallmark of PDAC is a tumor microenvironment dominated by stromal cells where tumor and immune cells are intermixed. The molecular characteristics and architecture of the tumor microenvironment conjure to inflict a heavy immune suppression that contributes to the dismal prognosis of this cancer. The development of new anti-cancer strategies that overcome this effect represents one of the main challenges in the field, for which it is essential to understand the molecular mechanisms behind the induction PDAC-induced tolerance. Changes in the glycosylation profile of cancer cells have been strongly associated with cancer progression and immune-modulation. The glycosylation signature effects inhibitory processes on many immune cells, that sense and develop inhibitory signals upon binding of the glycans through their lectins receptors. To deciphering the specific glycan signature of tumor cells, which we referred to as glyco-code, is important to understand how glycan–lectin circuits drive immune suppression in the tumor microenvironment. In this work, we performed a deep characterization of the glyco-code in pancreatic cancer by analyzing transcriptomic data of patient samples and cell lines, providing solid evidence of the clinical relevance of glycosylation and the induction of a tolerogenic microenvironment.

Our results show that the expression of glycosylation-related genes can be used for the stratification of patients in molecular subtypes with different clinical outcomes. The analysis of transcriptomic data of tissue from patients, and the subsequent validation in cell lines, allowed us to identify two subtypes of cancer cells that express specific glycan signatures and are associated with different ends of the Epithelial to mesenchymal transition continuum. We found that epithelial and mesenchymal cells are characterized by specific glycan signatures, particularly by the presence of the fucosylated carbohydrates. Our data show that these structures are expressed in cells with an epithelial phenotype, while they are absent in mesenchymal-like cells. The differential glycan signatures present in epithelial and mesenchymal cells impact their recognition by the immune system, in particular by cancer-induced Tumor associated macrophages. Indeed, while ligands of MGL are present in both kind of tumor types, ligands of the tolerogenic receptor DC-SIGN are expressed only in the epithelial cells. Interestingly, the glycan binding and triggering of DC-SIGN can clearly modulate the maturation of macrophages, by increasing the production of IL10, which may contribute to the tolerogenic microenvironment. In summary, we describe novel glycan-mediated tolerogenic circuits involved in PDAC tumor progression and the induction of its tolerogenic microenvironment.

Keywords: Glycosylation, Pancreatic cancer, Glyco-code.

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Rodriguez, E., Schetters, S. T. T. & Van Kooyk, Y. (2018). The tumour glyco-code as a novel immune checkpoint for immunotherapy. Nat Rev Immunol, 18, 204-211.

## A190 / The contrasting roles of IFNs in tumor growth control and immune checkpoint inhibitor immunotherapy efficacy

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Type I and II Interferons (IFNs) are pleiotrppic cytokines that play essential role in regulation of host immune response. It has been known for decades that the type II IFNg acts in concert with T lymphocytes to control tumor growth and thus is a key component of the host cancer immunosurveillance system. Type I IFN (IFN-I) has recently also emerged as a key component of the host cancer immunosurveillance. More importantly, it has recently been determined that the intrinsic signaling pathways of both Type I and II IFNs in tumor cells are essential for tumor response to immune checkpoint inhibitor (ICI) immunotherapy. Consistent with these findings, we observed that mice with deficiency of either IFNg or IFNAR1, the receptor for IFN-I, exhibit increased susceptibility to carcinoma-induced tumorigenesis and show impaired growth control of transplanted tumors. However, IFNAR1-sufficient tumors also grow significantly faster in IFNAR1-KO mice and in mice with IFNAR1 deficiency only in T cells (IFNAR1-TKO), suggesting that IFN-I functions in T cells to enhance host cancer immunosurveillance. Strikingly, tumor-infiltrating CTL levels are similar between tumor-bearing WT and IFNAR1-KO mice. Competitive reconstitution of mixed WT and IFNAR1-KO bone marrow chimera mice determined that IFNAR1-deficient naïve CTLs exhibit no deficiency in response to vaccination to generate antigen-specific CTLs as compared to WT CTLs. Gene expression profiling determined that Gzmb expression is down-regulated in tumor-infiltrating CTLs of IFNAR1-KO mice as compared to WT mice, and in antigen-specific IFNAR1-KO CTLs as compared to WT CTLs in vivo. Mechanistically, we determined that IFN-I activates STAT3 that binds to the Gzmb promoter to activate Gzmb transcription in CTLs. IFNAR1 expression level is significantly lower in human colorectal carcinoma tissues than in normal colon tissues. IFNAR1 protein is also significantly lower on CTLs from colorectal cancer patients than those from healthy donors. Paradoxically, sustained/chronic IFN signaling also promote tumor growth and confers tumor resistance to ICI immunotherapy. We observed that inhibition of IFN signaling with Roxilitinib selectively inhibits STAT1 and STAT3 activation and increases CTL infiltration to induce a Tc1/Th1 immune response to suppress tumor growth in a T cell-dependent manner in vivo. In addition, Ruxilitinib inhibits STAT3 activation in tumor cells to downregulate immune suppressive cytokines production to increase T cell activation and effector function. Taken together, our findings indicate that IFNs play a key role in host cancer immunosurveillance under physiological conditions and tumor cells may use down-regulating IFNAR1 on CTLs to suppress CTL effector function to evade host cancer immunosurveillance. However, under pathological conditions, chronic/sustained IFN signaling in tumor cells leads to immune suppression and targeting the chronic IFN signaling in tumor cells is an effective approach to enhance the efficacy of ICI immunotherapy.

Keywords: Type I interferon, CTLs, STAT3, Granzyme B.

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Type I IFNs activates STAT3 to regulate effector expression of cytotoxic T lymphocyte effectors to suppress tumor development. J Immunother Cancer. 2019 Jun 22;7(1):157.

## A191 / BET bromodomain proteins regulate T cell expression of multiple immune checkpoint molecules

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Tumors may be infiltrated with T cell subpopulations exhibiting minimal metabolic activity and proliferative capacity, which disable their ability to eliminate malignant cells by cytotoxicity, a state termed 'checkpoint inhibition'. In certain cancers, particularly melanoma, blockade of checkpoint inhibition has shown success to unleash the anti-tumor immunity of tumor-infiltrating lymphocytes (TILs). Yet, high variability in response rates among diverse patients remains a critical problem; insufficient progress has been made to understand mechanisms that resolve responders from non-responders. The transcriptional networks that regulate expression of immune checkpoint molecules are largely unknown, yet may provide crucial insight into pathways that could maximize therapeutic benefit. Here we used multiparameter flow cytometry to explore the expression of several important checkpoint inhibition molecules, focusing on the functional role of the Bromodomain and ExtraTerminal (BET) family of transcriptional co-regulators (BRD2, BRD3 and BRD4). We found that BET proteins regulate expression of inhibitory receptors PD-1, CTLA-4, TIM-3 and TIGIT in CD8+ T cells, yet the normal variation of BET profile is wide. Individual differences in expression of BET proteins and inhibitory receptors should inform clinical decision making for immune checkpoint approaches.

Introduction: We previously showed that BET proteins regulate tumor microenvironment pathways important for breast cancer progression [1] and proposed that immune checkpoints are transcriptionally regulated by BET proteins [2]. Yet small molecule pan-BET inhibitors like JQ1 ignore BET protein functional differences that may stratify responses [3]; for example, BRD2 functionally opposes BRD3 and BRD4 in epithelial-to-mesenchymal transition in breast cancer [4]. Therefore, personalized patient profiling of BET protein family members may reveal important individual-level differences that determine the success of immune checkpoint blockade. This approach may benefit breast cancer patients with chronic metabolic disease [5] that drives immune exhaustion in TILs. HYPOTHESES. BRD2, BRD3 and BRD4 separately regulate expression of inhibitory receptors PD-1, CTLA-4, TIM-3 and TIGIT in CD8+ T cells, and personalized patient profiles of BET gene expression determines individual signatures of immune checkpoint expression.

Method:. Human primary peripheral blood mononuclear cells (PBMCs) from normal healthy donors were activated ex vivo with plate bound anti-CD3 (10 ug/ml) and soluble CD28 (2 ug/ml) over 1–5 days, then multiparameter flow cytometry was performed with an LSR2 cytometer to assay expression of inhibitory receptors PD-1, CTLA-4, TIM-3 and TIGIT on T cell subsets. We leveraged our published 16-color panel for analysis of multiple inhibitory receptors [6]; live cell events were analyzed in FlowJo. Different small molecule inhibitors of BET protein expression, including the pan-BET inhibitor JQ1 and the BRD4-selective PROTAC degrader MZ-1, were used to identify BET protein-regulated targets. Individuals were profiled for their personalized expression profile of each BET gene by RT-PCR and BET protein by immunoblot, and PBMC profiles were matched to TILs in a preliminary cohort of patients with triple negative breast cancer.

Result:. We observed wide individual-level differences in expression of each BET family member in primary PBMCs. Pan-BET inhibition by JQ1 decreased expression of PD-1, CTLA-4, TIM-3 and TIGIT in activated CD8+ T cells. MZ1 was more potent to inhibit CTLA-4, indicating an essential role of BRD4 in its expression. Personalized patterns of BET gene expression and inhibitory receptor expression on PBMCs generally matched TIL patterns in patients with triple negative breast cancer.

Conclusion. Personalized BET profiles define individual responses to antibody blockade of immune checkpoints; blood biomarkers for potential response could have value in breast cancer.

Keywords: BET bromodomain, multiparameter flow cytometry, personalized medicine, breast tumor T cell infiltrates.

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## A192 / Total neoadjuvant therapy with FOLFIRINOX in combination with losartan followed by chemoradiotherapy for locally advanced pancreatic cancer: A phase 2 clinical trial.

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Importance: Patients with locally advanced pancreatic cancer have historically poor outcomes. Evaluation of a total neoadjuvant approach is warranted.

Objective: To evaluate the margin-negative (R0) resection rate of neoadjuvant FOLFIRINOX (fluorouracil, leucovorin, oxaliplatin, and irinotecan) and losartan followed by chemoradiotherapy for locally advanced pancreatic cancer.

Design, setting and paticipants: A single-arm phase 2 clinical trial was conducted at a large academic hospital from August 22, 2013, to May 22, 2018, among 49 patients with previously untreated locally advanced unresectable pancreatic cancer as determined by multidisciplinary review. Patients had Eastern Cooperative Oncology Group performance status 0 or 1 and adequate hematologic, renal, and hepatic function. Median follow-up for the analysis was 17.1 months (range, 5.0-53.7) among 27 patients still alive at study completion. INTERVENTIONS: Patients received FOLFIRINOX and losartan for 8 cycles. Patients with radiographically resectable tumor after chemotherapy received short-course chemoradiotherapy (5 GyE × 5 with protons) with capecitabine. Patients with persistent vascular involvement received long-course chemoradiotherapy (50.4 Gy with a vascular boost to 58.8 Gy) with fluorouracil or capecitabine. MAIN OUTCOMES AND MEASURES: R0 resection rate.

Results: Of the 49 patients (26 women and 23 men; median age 63 years [range, 42-78 years]), 39 completed 8 cycles of FOLFIRINOX and losartan; 10 patients had fewer than 8 cycles due to progression (5 patients), losartan intolerance (3 patients), and toxicity (2 patients). Seven patients (16%) had short-course chemoradiotherapy while 38 (84%) had long-course chemoradiotherapy. Forty-two (86%) patients underwent attempted surgery, with R0 resection achieved in 34 of 49 patients (69%; 95% CI, 55%-82%). Overall median progression-free survival was 17.5 months (95% CI: 13.9-22.7) and median overall survival was 31.4 months (95% CI, 18.1-38.5). Among patients who underwent resection, median progression-free survival was 21.3 months (95% CI, 16.6-28.2), and median overall survival was 33.0 months (95% CI, 31.4 to not reached).

Conclusion and relevance: Total neoadjuvant therapy with FOLFIRINOX, losartan, and chemoradiotherapy provides downstaging of locally advanced pancreatic ductal adenocarcinoma and is associated with an R0 resection rate of 61%.

Trial registration: ClinicalTrials.gov identifier: NCT01821729.

Keywords: chemoradiation, Resection, desmoplasia, angiotensin receptor blockers.

References:
ClinicalTrials.gov identifier: NCT01821729.

## A193 / The role of CD163-positive macrophages in human and murine sarcoma - a new target for anti-cancer therapy

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Tumor-associated macrophages (TAMs) play a pivotal role in tumor progression, which affect tumor growth, tumor angiogenesis, immune suppression, and metastasis. Most human studies disclosed that a positive correlation between TAM density and a poor prognosis of the patients. Studies focusing on macrophage phenotypes emphasized the protumoral role of M2-like anti-inflammatory macrophages in many types of human malignancies including epithelial and non-epithelial tumors. CD163 is one of the most widely used markers for M2-like macrophages. CD163 is a cell surface glycoprotein receptor with a molecular weight of 130kDa and was first found to be a receptor of the hemoglobin and haptoglobin complex. In the present study, we analyzed 62 human cases of “undifferentiated pleomorphic sarcoma (UPS)”. As the fact that this tumor was misnamed as “malignant fibrous histiocytoma” indicates, many macrophages were detected among tumor cells. We analyzed the density of M2-like macrophages using anti-CD163 and compared with that of total macrophages detected by Iba1 antibody, one of the pan-macrophage markers. The median densities of CD163-positive and Iba1-positve TAMs were 406/mm2 and 683/mm2, respectively, and median percentage of CD163-positive cells in Iba1-postive cells was 78%. A high density of CD163-positive TAMs was significantly associated with higher clinical stage. Notably, a high percentage of CD163-positive TAMs was associated with decreased overall survival and higher histological grade. Whereas there was no significant association between the density of Iba1-positive total TAMs and all clinical parameters. To elucidate the role of CD163 molecule we evaluated transplanted murine sarcoma in CD163-deficient mice. The results showed that tumor development and lung metastasis were significantly suppressed in CD163-deficient mice compared with that in wild-type mice. These observations indicated the significance of CD163 in the protumor functions of TAMs. In further in vitro experiments, coculture with wild-type peritoneal macrophages significantly increased proliferation of murine sarcoma cells but decreased in the presence of CD163-deficient macrophages. Cytokine analysis revealed that production of IL-6 and CXCL2 was suppressed in CD163-deficient macrophages in comParison to wild-type macrophages. Silencing of IL-6 but not CXCL2 abrogated macrophage-induced proliferation of mouse sarcoma cells indicating IL-6, rather than CXCL2, derived from macrophages is involved in tumor proliferation under the coculture condition. Taken together, our results indicates that CD163 is involved in protumoral activation of macrophages and subsequent development and progression of tumors in mice and humans. Current study of human UPS and murine sarcoma experiments indicates that CD163 molecule and CD163-positive TAMs are considered as new targets for anti-cancer therapy.

Keywords: tumor-associated macrophages, CD163, undifferenti-

ated pleomorphic sarcoma.

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## A194 / Expression of PD-1 in cancer cells

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The purpose of the study was to evaluate the expression of various immune-inflammation-related factors including PD-1 and PD-L1 in various cancer cell lines (HeLa - cervical cancer cells, MCF-7 - breast cancer cells, K-562 - chronic myeloid leukemia cells).

The evaluation was performed using RT PCR for all factors. All studied cell lines have shown relatively high expression of PD-1. Notably, expression of PD-1 in all cell lines was greater than expression of PD-L1.

Relative expression of PD-1 and PD-L1 were for HeLa cells: 4, 6 3 2.1% and 0, 5 3 0, 3%, respectively; for MCF-7 cells: 5, 4 3 1, 1 and 1, 6 3 0, 6%; for K-562 cells: 18, 0 3 3, 0% and 1, 2 3 0, 1% (mean 3 S.D., GAPDH as the internal standard).

Inflammation is linked to immune tolerance. Low-grade inflammation mediates immune tolerance in normal tissues (e.g. in pregnancy, gut tolerance, etc.) as well as in cancer. Cancer cells per se can promote inflammatory microenvironment which facilitates immune exhaustion. One of examples is detection of PD-1 expression in cancer cells. It should be noted that expression of PD-1 by cancer cells has a dual role. According to the latest data in some cases (e.g. in melanoma) blocking of cancer cell-intrinsic PD-1 can help to limit tumor growth. In contrast to this in other cases (e.g. in lung cancer) blocking of cancer cell-intrinsic PD-1 can promote tumor growth. That's why it is important to study PD-1 signaling pathways in various types of cancer cells. This should be taken into account in checkpoint inhibition therapy as a possible biomarker capable of predicting which patients are most likely to respond to anti PD-1 therapy.

Keywords: PD-1, Immune tolerance, Inflammation, Immune checkpoints.

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## A195 / CD24 signaling through macrophage Siglec-10 is a new target for cancer immunotherapy

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Ovarian cancer and triple-negative breast cancer (TNBC) are among the most lethal diseases affecting women, with few targeted therapies and high rates of metastasis. Here we show that CD24 can be the dominant innate immune checkpoint in ovarian cancer and breast cancer, and is a new, promising target for cancer immunotherapy. Cancer cells are capable of evading clearance by macrophages through the overexpression of anti-phagocytic surface proteins, called "don't eat me" signals, including CD47 (1), programmed cell death ligand 1 (PD-L1) (2), and the beta-2 microglobulin subunit of the major histocompatibility class I complex (B2M) (3). Monoclonal antibodies which antagonize the interaction of "don't eat me" signals with their macrophage-expressed receptors have demonstrated therapeutic potential in several cancers (4-5). However, variability in the magnitude and durability of the response to these agents has suggested the presence of additional, as yet unknown, "don't eat me" signals. Here we demonstrate a novel role for tumor-expressed CD24 in promoting immune evasion through its interaction with the inhibitory receptor, Sialic Acid Binding Ig Like Lectin 10 (Siglec-10), expressed by tumor-associated macrophages (TAMs). We observe that many tumors overexpress CD24 and that TAMs express high levels of Siglec-10. Both genetic ablation of CD24 or Siglec-10, and monoclonal antibody blockade of the CD24–Siglec-10 interaction, robustly augment the phagocytosis of all CD24-expressing human tumors tested, in many cases outperforming CD47 blockade. Genetic ablation as well as therapeutic blockade of CD24 resulted in a macrophage-dependent reduction of tumor growth and extension of survival, in vivo. These data highlight CD24 as a highly-expressed, anti-phagocytic signal in several cancers and demonstrate the therapeutic potential for CD24-blockade as cancer immunotherapy.

Keywords: Immunotherapy, Macrophages, Immunosurveillance, Translational research.

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## A196 / Inhibition of Janus Kinase 3 (JAK3) improves anti-tumor immunity and inhibits tumor outgrowth

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An immune suppressive tumor microenvironment as well as functional impairment of T-cell mediated anti-tumor immunity are features that define most types of cancer and are factors that underlie resistance to anti-cancer therapies. Therefore, defining novel therapeutic targets that alleviate immune suppression and improve T-cell phenotype may enhance anti-tumor immunity and ultimately patient survival. A tyrosine kinase that is expressed both in tumor-associated macrophages as well as in T cells is Janus Kinase 3 (JAK3), which is essential for the common cytokine receptor gamma chain. In macrophages, JAK3 is involved in interleukin 4 (IL-4) receptor signaling which induces immune suppressive "M2" macrophage polarization whereas JAK3 is involved in IL-2 receptor mediated induction of T-cell proliferation. Modulation of JAK3 by low-grade inhibition could therefore inhibit "M2" macrophage polarization and modulate T-cell phenotype. Therefore, we aimed to investigate the effect on macrophage polarization, T-cell phenotype and anti-tumor immunity of a recently described specific JAK3 inhibitor (PF-06651600). To exploit the direct effect of JAK3 inhibition on macrophages and T cells, we assessed levels of immune suppressive "M2" macrophage surface markers as well as phosphorylation levels of signaling molecules involved in various T-cell signaling pathways after in vitro incubation of macrophages and T cells with PF-06651600. The effect on anti-tumor immunity was investigated in a mesothelioma model by treating C57/BL6 mice or CBA/J mice inoculated with respectively AE17 or AC29 tumor cells with PF-06651600 dissolved in drinking water ad libitum (5 mg/kg). In vitro incubation of murine and human macrophages with PF-06651600 demonstrated that JAK3 inhibition prevents IL-4 mediated "M2" macrophage polarization at nanomolar concentrations as observed by decreased expression of immune suppressive molecules on macrophages (PD-L1/2, IDO and IL-10). In addition, JAK3 inhibition specifically inhibited STAT5 phosphorylation in T cells, which induced decreased T-cell activation and proliferation only at high concentrations. However, in an immune competent murine tumor model,

low-dose continuous administration of JAK3 inhibition in drinking water (5 mg/kg) resulted in improved CD8+ T-cell phenotype as indicated by enhanced proliferation (mean Ki67+: 9.4% vs 32%, p<0.05) and activation (CD69+: 5.2 vs 16%, p<0.05) as well as improved tumor responses as observed by a reduced tumor weight compared to untreated mice (mean tumor weight: 0.9 vs 1.7 grams, respectively, p<0.05). Furthermore, JAK3 inhibition combined with tumor vaccination yielded superior anti-tumor effects as compared to no or either treatment alone. These effects were not due to direct anti-tumor effects of PF-06651600, as in vitro tumor cell proliferation was not perturbed by the addition of PF-06651600 to the culture. Collectively, these data indicate that low-grade modulation of JAK3-associated signaling pathways by using specific JAK3 inhibition reduces "M2" macrophage polarization and improves T-cell immunity observed by the improved T-cell phenotype and reduced tumor load. These findings pave the way for further investigation of JAK3 inhibition in improving anti-tumor immunity and provide novel opportunities for therapy development.

Keywords: T cells, Macrophages, Tumor microenvironment, JAK3.

## A197 / The tumor glycosphingolipid repertoire orchestrates HLA class I-mediated immune responses

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HLA class I (HLA-I) proteins drive immune responses by presenting antigen-derived peptides to cognate CD8+ T cells. Molecular loading of immune stimulatory peptides into HLA-I proceeds through an elaborate process involving many steps, which can be exploited for immune escape by tumors. Here, we report an unexpected new regulatory layer of HLA-I biology. Using iterative genome-wide haploid genetic screens, we find that the intramembrane cleaving protease SPPL3 enhances HLA-I antigen presentation by inhibiting the activity of B3GNT5, an enzyme of the glycosphingolipid (GSL) synthesis pathway. GSLs generated by B3GNT5 shield HLA-I from interacting with other proteins and diminish CD8+ T cell activation. Glioblastoma and AML tumors overexpressing B3GNT5 GSLs also show an impaired capacity of HLA-I to activate T cells which can be rescued by GSL deletion, suggesting that GSLs are involved in escape from T cell surveillance. The immune modulatory effect of B3GNT5 GSLs can also be reversed through inhibition of their synthesis using clinically approved drugs. Our findings demonstrate that the composition of the GSL repertoire determines the efficacy of antigen presentation and represents a potential treatment target in cancer.

Keywords: HLA class I antigen presentation, Genome wide screen, Glycolipids, Glioblastoma.

## A198 / Selective PD-L1 blockade in the tumor-draining lymph node induces effective anti-tumor T-cell immunity

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Checkpoint immunotherapy for solid tumors is generally thought to act directly in the tumor microenvironment. The relevance of tumor-draining lymph nodes (TDLNs), however, remains largely unknown. In TDLNs circulating T cells are exposed to tumor-antigens in the absence of a suppressive tumor microenvironment. Therefore, TDLNs could offer a central hub for the generation of anti-tumor immune responses. Dissecting the specific role of TDLNs in immunotherapy has proven difficult as pharmacologic or genetic Methods of ablation affect the entire host including the tumor. More in-depth analysis and specific targeting of the TDLN could offer novel insights into immunotherapy mode of action and benefit future immunotherapeutic treatment strategies.

To evaluate whether TDLNs harbor tumor-antigen specific T cells susceptible to immune-checkpoint blocking antibodies, we evaluated the expression of multiple co-inhibitory receptors and ligands on leukocyte subsets in TDLNs and non-TDLNs in various solid tumor models using multicolor flow-cytometry. Furthermore, a method was developed whereby a titrated low-dose concentration of anti-PD-L1 antibodies was administered intrapleurally. As tumors (including MC38-colon, AC29- and AE17-mesothelioma tumors) were located intraperitoneally, this enabled for specific targeting of the (mediastinal) TDLN, without translocation of the antibody to the tumor. Using this method, we evaluated potential efficacy of TDLN-directed anti-PD-L1 immunotherapy and assessed mechanisms of action using flow- and histo-cytometry on LNs, peripheral blood and tumor tissue.

We found that TDLNs from solid tumors were enriched for tumor antigen-specific PD-1+ T cells, the frequency of which correlated with tumor-antigenicity, independently of tumor type and anatomical location. In parallel, TDLN myeloid cells including macrophages and type 2 conventional dendritic cells (cDC2) highly expressed PD-L1 as compared their non-TDLN counterparts, approaching PD-L1 expression in tumors. TDLN-directed delivery of PD-L1-blocking antibodies was feasible, with particular binding to LN cDC2s and macrophages irrespective of mode of antibody delivery. Specific TDLN-targeting resulted in PD-1+ T cell mobilization and effective delay in tumor outgrowth in several mouse models. TDLN-local or systemic anti-PD-L1 treatment strongly upregulated co-inhibitory immune receptors on CD8+ tumor-infiltrating T cells, with LN-local administration specifically leading to co-expression of all receptors whereas systemic treatment did not. Finally, several intervention and imaging studies pointed to TDLN-resident type 2 conventional dendritic cells (cDC2s), but not macrophages as mediators of local T-cell inhibition.

We have for the first time shown that specific targeting of the PD-1/PD-L1 axis in TDLNs contributes to primary immunotherapy efficacy. Our findings reveal a critical role of TDLNs in cancer immunity, and pave the way for further in-depth analysis of TDLN function for the benefit of effective immunotherapy development.

Keywords: T cells, Tumor-draining lymph node, Immunotherapy, PD-1/PD-L1.

## A199 / Transcriptomic profile of human keratinocytes in the presence of a novel synthetic retinoid-polyamine conjugate

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We tested the effect of a novel synthetic N1, N12-Bis(all-trans-retinoyl)spermine retinoid-spermine conjugate (RASP; International Patent Publication Number WO 2004/018001 A1) on HaCaT cell line. Recently, novel synthetic retinoids with elaborate efficiency and minimum side effects have emerged as novel drugs. RASP has an established anti-cancer and anti-proliferative activity when tested in several cancer cell lines and moreover, exhibits limited toxicity and teratogenicity in rats. The purpose of the present study was to assess the effect of RASP on gene expression of Ha-

CaT cells, a model of human epidermis. In addition, we measured the expression of miRNAs using NGS analysis and we correlated the molecular pathways that are affected and exhibit miRNA-mediated regulation, in order to evaluate the potency of RASP as a leading compound for skin cancer.

Expression profile of transcripts from RASP treated (1μM RASP; IC50) and untreated cells was analysed using DNA microarrays (One Array) on a Perkin Elmer platform (ScanArray Express). The small RNAs fraction (<200 nt) was isolated with MiRVana (Ambion) and used for NGS analysis on an IonTorrent PGM platform according the manufacturer's instructions. Bioinformatics analysis was performed using InSyBio Suite (www.insybio.gr) for functional enrichment analysis and the construction of miRNA-mRNA networks. The most significant alterations both in mRNAs and miRNAs were verified by qRT-PCR. Apoptosis in various concentrations of RASP was monitored by observing mitochondrial integrity via confocal microscopy and cell cycle progression by FACS analysis.

The effect of RASP on HaCaT cells resulted in 1437 downregulated and 416 upregulated genes during the mRNA expression analysis. In addition, 35 upregulated miRNAs targeting 1159 of the downregulated genes and 25 downregulated miRNAs targeting 336 upregulated genes were identified after target prediction analysis. The analysis using the InSyBio Suite offers a unique miRNA target-prediction pipeline, which results in scored miRNA target sites in mRNAs with over 95% accuracy. Protein-protein interactions analysis was performed using the miRNA target-predicted genes, resulting in 31 downregulated and 2 upregulated significant genes involved in statistically significant KEGG pathways like: NF-kappa B signalling, RIG-I-like receptor signalling pathway, T cell receptor signalling, FoxO signalling, MAPK signalling and microRNAs in cancer. Several genes affect the induction of apoptosis and interfere with different cell cycle phases. FACS analysis showed significant cell cycle arrest in G2 phase and DNA damage. Cell cultures at various steps and conditions showed extensive mitochondrial breakdown and DNA fragmentation which is also supportive for the apoptotic path of cells after exposure to RASP. RASP is a new synthetic retinoid-based compound with anti-proliferative and anti-tumorigenic effects. Our analysis showed that many key miRNAs that are deregulated in melanoma and target important genes which regulate key components of the cell cycle and apoptosis are downregulated in the presence of RASP. Our transcriptomic analysis taken together with our previous studies in cell lines and animal models strongly support the anti-proliferative and anti-tumorigenic effects of RASP which could also be extended in studies concerning treatment of melanoma and skin cancer. RASP could provide the basis for further development of improved synthetic retinoid analogues used alone or in combination therapy, without the deleterious effects of previous compounds in use.

Keywords: Retinoids, Skin cancer, Transcriptomics, miRNAs.

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## A200 / Harnessing lymphoid neogenesis for prognosis and treatment of solid tumors

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Lymphoid neogenesis generates ectopic lymphoid organs called tertiary lymphoid structures (TLS) in chronically inflamed tissues including cancer. TLS are sites of naïve T and B cell activation and their presence correlates with prolonged survival in various cancer types. This suggests that TLS contribute to anti-tumor immunity and that TLS induction in tumors could be a novel immunotherapeutic approach. However, molecular and cellular mechanisms of TLS development in cancer or how TLS contribute to survival are largely not understood. Here we used a quantitative pathology approach based on multi-parameter immunofluorescence and multi-spectral microscopy to analyze TLS in cohorts of lung squamous cell carcinoma (n=138), colorectal cancer (n=111), bladder cancer (n=33) and renal cancer (n=50). We discovered that TLS development followed a common maturation sequence that culminated with the activation of a germinal center (GC) reaction in all analyzed tumor types. The extent of TLS development, however, differed significantly between the tumor types: lung and bladder cancer had the highest, colorectal cancer had an intermediate and renal cancer had the lowest number of TLS. We found that the number of mature GC-positive TLS and not immature TLS was an independent prognostic factor for survival in untreated patients of all tumour types except renal cancer. Our analysis of neoadjuvant chemotherapy-treated lung and bladder cancer cohorts demonstrated that the prognostic significance of TLS was lost in these patients. We observed that total TLS numbers were not affected but GC formation was severely impaired after chemotherapy. Corticosteroids have a broadly anti-inflammatory effect and are commonly used to manage side effects of chemotherapy in lung and bladder cancer. To determine the effects of corticosteroids on cancer-associated TLS development we analyzed chemotherapy-naïve lung cancer patients that received corticosteroids to treat other comorbidities such as chronic obstructive pulmonary disease and others prior to surgery. We observed a significant reduction of TLS numbers and GC development in these patients compared to patients without any steroid therapy. Together, these results suggest that GC development either represents the relevant TLS function in anti-tumour immunity or indicates to an immune promoting microenvironment that can be counteracted by corticosteroids. To address these questions experimentally, we established an intranasal application protocol to induce TLS in the lungs of mice. We identified a combination of inflammatory stimuli that lead to GC-positive TLS development in naive lungs following the same maturation sequence as in cancer patients. We demonstrated a negative impact of low dose dexamethasone on TLS and GC development in this model. In summary, the number of GC-positive TLS is an independent prognostic marker in lung, bladder and colorectal cancer but not renal cancer patients. Renal cancer has the lowest number of TLS among the analyzed tumor types and insufficient number of GC-positive TLS might explain the lack of prognostic relevance. Corticosteroids impair TLS and GC development in cancer patients and in the established experimental model of lung TLS induction. This model recapitulates cancer-associated TLS maturation stages observed in patients and can be used to study the role of TLS in anti-tumor immunity.

Keywords: tertiary lymphoid strcutres, ectopic germinal center, tumor microenvironment, corticosteroids.

## A201 / Citrullinated glucose-regulated protein 78 is a candidate target for cancer immunotherapy

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Post translational modification of proteins plays a significant role in the progression of autoimmune disease. In particular the modification of arginine to citrulline which is mediated by PAD enzymes. This modification is increased during cellular stress (autophagy) which permits the presentation of modified epitopes upon MHC class II molecules for recognition by CD4 T cells. Citrullination also occurs in tumour cells as a result of continuous environmental stresses and increased autophagy. We have shown in animal models the efficient stimulation of citrulline specific CD4 T cells and their targeting of tumors resulting in dramatic elimi-

nation/regression and enhanced survival. The ER chaperone glucose-regulated protein 78 (GRP78) is known to also be required for stress-induced autophagy and is directly linked to autophagosome formation. GRP78 is known to be highly expressed by many tumor types. In this study we investigate the potential of targeting citrullinated GRP78 for cancer therapy. We select five peptides based upon predicted MHCII binding scores and show the identification CD4 T cell responses to one citrullinated GRP78 epitope that are restricted through HLA DP4, HLA-DR4 and HLA-DR1 alleles. Citrulline specific CD4 responses to this epitope mediate efficient therapy of established B16 melanoma tumors in HLA-DP4 ($p<0.0001$) and HLA-DR4 ($p=0.0013$) transgenic mouse models. Finally we demonstrate the existence of a repertoire of responses with proliferative capacity to the citrullinated GRP78 peptide in healthy individuals ($p=0.0023$) with 13/17 (76%) individuals showing a response to the peptide. In contrast, only 9/22 (41%) of cancer patients showed responses to the same peptide and these were of lower frequency suggesting the response may be attenuated in cancer patients ($p<0.05$). We propose that vaccination against citrullinated GRP78 may help overcome this and provide an application to tumor therapy.

Keywords: glucose-regulated protein 78, citrullination, CD4 T cell.

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## A202 / Time-dependency of PD-L1 targeting antitumor efficacy against PD-L1 deficient tumors

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PD-L1/PD-1 signaling plays a critical role in tumor evasion, with dramatic responses of therapy-refractory cancers to therapeutic blockade targeting this signaling axis. However, additional cellular and molecular mechanistic understandings must be sought to further improve current immune checkpoint blockade (ICB) therapies and to circumvent undesirable side effects. Clinical specimens revealed that most pediatric cancers do not express PD-L1 or express it at a low level, causing skepticism for potential efficacy of ICB in these tumors. Previously, we reported that Cyclin-dependent Kinase 5 (Cdk5) regulates tumor Programmed Death-Ligand 1 (PD-L1) in response to interferon-gamma (IFNg) in a murine medulloblastoma (MB) model derived from Ptch+/-/p53-/- mice. Cdk5def MB solicits CD4+ T cell-dependent rejection linked to enhanced tumor microenvironment (TME) IFNg contents, increased PD-L1 expression in lymphoid and myeloid cells, and a blunted tumor PD-L1 expression in response to IFNg in p53 and SHH altered MB and rhabdomyosarcoma. Now using a high potency, orally available, non-selective Cdk5 inhibitor, CYC065, we observed significant reduction in MB tumor growth in vivo, with resultant explanted tumors showing epigenetically altered PD-L1, MHC-I and MHC-II responses to IFNg in vitro in the absence of further Cdk5 inhibition. As Cdk5 disruption reduces tumor PD-L1 expression, we investigate whether such reduction is directly responsible for enhanced immunity. To interrogate this, we genetically deleted PD-L1 in MB, and observed a time-dependent, strong efficacy of anti-PD-L1 mediated ICB in PD-L1def MB. 5x10e4 PD-L1def or Cdk5def MB resulted in a 50% tumor incidence as compared to 100% tumor incidence with wild type MB. Weekly administration of anti-PD-L1 antibody to mice bearing PD-L1def MB starting at 7 days following tumor inoculation resulted in 100% tumor rejection, while injection of antibody treatment starting on day 0 of tumor inoculation resulted in only 30-50% tumor-free incidence. Our data support the view that the absence of tumor PD-L1 does not preclude the potential utility of checkpoint blockade, and alternative cellular mechanisms of how PD-L1/PD1 interaction enhances immune function among non-tumor cells in a time-dependent manner need be further examined. Ongoing in vitro co-culture experiment shows a dose-dependent inhibition of T cell activation upon IFNg receptor signaling upon early PD-L1 blockade, potentially providing a molecular explanation for the observed time-dependency of ICB efficacy against PD-L1def tumor. Our observations establish a functional link between an increasingly important serine/threonine kinase in cancer to immune checkpoint regulation and other aspects of tumor immunity, provide the scientific rationale to carefully time the application of ICB in PD-L1lo tumors, and allow the pursuit of pharmacologic Cdk5 inhibitor to modulate tumor sensitivity for immunotherapy.

Keywords: T cell activation, medulloblastoma, time-dependency, PD-L1 deficient.

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## A203 / A small molecule triple-negative breast cancer immunotherapy that targets the Liver-X-Receptor.

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Triple negative breast cancer (TNBC) affects 10-20% of the 1 in 8 women diagnosed with breast cancer every year. TNBC is the most aggressive form of breast cancer that unfortunately cannot be treated with hormonal or HER2 targeted therapies, leaving patients with limited treatment alternatives. Fortunately, TNBC responds to chemotherapy if caught early. However, treatment for recurrent TNBC is limited, therefore TNBC continues to present a formidable clinical challenge. Cancer immunotherapy, which stimulates the immune system to destroy tumors has revolutionized cancer treatment by producing astounding clinical results in late stage cancers. There is only FDA-approved immunotherapy for TNBC, Atezolizumab for patients with high PD-L1 expression, which unfortunately only marginally improves patient survival (~2 months). As a result, TNBC is still considered to have limited responsiveness to immune checkpoint-blockade. Therefore, research efforts centered on developing effective immunotherapies for TNBC are of prime importance and should help save the lives of the thousands of patients that succumb to TNBC annually.

Tight coordinated regulation of lipid and glucose metabolism in immune cells is essential to mounting an effective anti-tumor immune response. It has been extensively demonstrated that aberrant lipid and glucose metabolite production are central to breast cancer etiology and treatment resistance. The Liver-X-Receptors (NR1H2, NR1H3) are nuclear receptors that function as key regulators of lipid metabolism and are potent attenuators of immune activity. In solid and hematological malignancies cancer cells have been shown to produce lipid metabolites that inhibit anti-tumor immune function by activating LXRs in immune cells. We hypothesized that TNBC tumor lipid metabolites drive tumor immune evasion by activating LXR in tumor infiltrating immune cells which inhibits anti-tumor immune activity and disrupts immune cell metabolic function.

In this study we highlight that TNBC cells produce lipid metabolites, that activate LXR to suppress immune function in a pleiotropic manner. Using LXR-driven luciferase reporter assays, we show that TNBC cells produce lipids that act as LXR agonists. In accord with this TNBC-lipids inhibited anti-tumor (M1) macrophage differentiation and suppressed dendritic cell C-C chemokine receptor 7 (CCR-7) expression, vital for DC lymph-node homing and antigen presentation. LXR activation using TNBC-lipids or the synthetic LXR ligand GW3965 directly stimulated Treg differentiation and expression of the checkpoint blockade ligand cytotoxic-T lymphocyte associated protein-4 (CTLA-4). We also observed that TNBC lipids inhibited CD8+ T-cell differentiation and activation indicated by reduced expression of IFNy and granzyme B. Secondly, we demonstrate in vitro and in vivo that TNBC-lipid induced immune suppression can be reversed by pharmacological inhibition of LXR activity using the LXR inverse-agonist SR9243; a compound we have developed and characterized previously. In vitro, SR9243 stimulated CD8+T-cell differentiation and

activation, repressed Treg differentiation and promoted M1 and suppressed pro-tumor (M2) differentiation. Importantly, SR9243 potently induced immune-mediated tumor destruction of E0771 TNBC tumor allografts in a CD8+ T-cell dependent manner, by repressing LXR activity in immune cells. Lastly, through extracellular flux analysis and mitochondrial activity profiling we have determined that LXR activation severely disrupts mitochondrial function in CD8+ T-cells. Our discoveries implicate LXR as a central mediator of TNBC immune evasion and as a promising treatment target for the development of novel small molecule immunotherapies for TNBC.

Keywords: The Liver-X-Receptor, Triple-Negative Breast Cancer, Cancer Immunotherapy, Tumor Microenvironment, Tumor Lipogenesis, Immune Cell Metabolism.

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## A204 / Bystander IFN-g activity promotes widespread and sustained cytokine signaling altering the tumor microenvironment

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The cytokine IFN-g produced by tumor-reactive T cells is a key effector molecule with well characterized pleiotropic effects during anti-tumor immune responses. While IFN-g production is a transient event targeted at the immunological synapse, how IFN-g acts in time and space within the tumor microenvironment has remained elusive. Here, we report that T cell-derived IFN-g profoundly impacts tumor and infiltrating immune cells. By creating mosaic tumors containing antigen-positive and negative cells, we demonstrated that IFN-g production required local antigen recognition but that IFN-g diffused extensively to act in antigen-negative tumor areas. Using intravital imaging and a reporter for STAT1 translocation, we provide evidence that T cells can mediate sustained IFN-g signaling, even in distant tumor cells. Furthermore, we show that tumor phenotypic alterations required several hours of exposure to IFN-g, most likely due to a positive feedback loop mediated by STAT1 and IRF8. This temporal requirement likely disfavors local IFN-g activity over diffusion and bystander effects. Finally, single-cell RNA-seq data from melanoma patients also suggested bystander IFN-g activity in human tumors. Our results indicate that tumor-reactive T cells act collectively to create large and sustained cytokine fields that profoundly modify the tumor microenvironment.

Keywords: Interferon gamma, Cytokine diffusion, Intravital imaging.

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## A205 / PRAME, tumor-associated antigen and modulator of anti-tumor immunity

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PReferentially expressed Antigen in Melanoma (PRAME) is a cancer testis antigen that has shown great potential as a prognostic biomarker and immunotherapeutic target in solid cancers. PRAME expression is upregulated in various solid tumor types and has been associated with more advanced disease and metastasis. However, its function in normal and neoplastic cells remains poorly understood. In our recent work, we have demonstrated that PRAME can mediate the epithelial-to-mesenchymal transition, and hence promote tumor progression (Al-Khadairi et al, 2019). To our best knowledge, however, it is unknown how PRAME interacts with other players within the tumor microenvironment, and whether PRAME is involved in anti-tumor immunity and/or response to cancer immunotherapy. In the present study, we investigated the effect of PRAME overexpression in tumor cells on the expression of immune-related genes and T cell activity in vitro. For this purpose, we generated a PRAME overexpressing MDA-MB-468 breast cancer cell line in addition to the parental cell line. Co-culture of T lymphocytes with PRAME-overexpressing cancer cells significantly reduced T cell activation, as measured by IFN-γ ELISpot. Moreover, we observed a reduction in cancer cell killing of PRAME-overexpressing cells compared to the parental cells. Interestingly, we found that the secretion of IFN-γ was reduced in direct and indirect co-culture systems, suggesting that PRAME might be involved in the regulation of secreted pro- and anti-inflammatory mediators in addition to affecting direct cell-cell contact with T lymphocytes. Analysis of the mRNA expression of various immune-related genes in the PRAME-overexpressing cancer cells revealed a downregulation of molecules involved in antigen presentation, immune cell recognition, macrophage activation, and immune-mediated tumor cell killing such as β2-microglobulin, HLA-B, MICA, CSF1, TNFSF10, IRF1 and FASLG to name a few. On the other hand, we observed a marked increase in the expression of the epigenetic regulator AICDA, the immunosuppressive molecule IDO1, and numerous pro-tumorigenic factors. We did not observe major changes in the expression of immune checkpoint ligands. In addition, a comprehensive analysis of secreted cytokines revealed PRAME-mediated downregulation of cytokines regulating T cell differentiation, activation and migration (e.g. IFN-γ, IL-18, CCL4, CCL19); as well as of cytokines involved in macrophage activation and migration (e.g. MCP-1, MIF, MIC-1), and in chemotaxis of neutrophils and natural killer cells (e.g. CCL4, CCL7, MCP-1).

In conclusion, our findings suggest a substantial immune modulation of the tumor microenvironment by PRAME-overexpressing cancer cells.

Our current results, together with our previous work, suggest that PRAME is a tumor-associated antigen that can promote tumorigenesis and to some extent dampen the anti-tumor immune response through dysregulation of immune-related genes in favor of an immunosuppressive tumor microenvironment. These findings support the potential of PRAME-specific immunotherapy to specifically target tumor cells and concomitantly alleviate inhibition of the anti-tumor immune response.

Keywords: PRAME, cancer testis antigen, immunosuppression, T cell activation.

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## A206 / CD29 marks superior cytotoxic human T cells

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Cytotoxic CD8+ T cells can effectively kill target cells by producing cytokines, chemokines and granzymes. Expression of these effector molecules is however highly divergent, and tools that identify and pre-select potent killer cells are lacking. Human CD8+ T cells can be divided into IFN-gamma and IL-2 producing cells. Unbiased transcriptomics and proteomics analysis on cytokine-producing fixed CD8+ T cells revealed that IL-2+ cells produce helper cytokines, and that IFN-gamma+ cells produce cytotoxic molecules. IFN-gamma+ T cells could be identified with the surface marker CD29 already prior to stimulation. CD29 also marked T cells with cytotoxic gene expression from different tissues in single-cell RNA-sequencing data. Notably, the cytotoxic features of CD29+ T cells were maintained during cell culture, suggesting a stable phenotype. Pre-selecting CD29-expressing MART1 TCR-engineered T cells potentiated the killing of target cells. We therefore propose that CD29 expression can help evaluate and select for potent therapeutic T cell products.

Keywords: CD8 T cells, cytotoxicity, transcriptomics and proteomics on Fixed cells.

## A207 / Resident memory CD8+ T cells increase the breadth of anti-tumor T cell immunity through dendritic cells

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Tissue-resident memory CD8+ T (Trm) cells mediate potent local innate and adaptive immune responses and play a central role against solid tumors. However, whether Trm cells cross-talk with dendritic cells (DCs) to support anti-tumor T cell immunity remains unknown. Here we show that antigen-specific activation of skin Trm cells led to maturation and migration to draining lymph nodes of cross-presenting dermal DCs. Tumor rejection mediated by Trm cells triggered the spread of cytotoxic CD8+ T cell responses against multiple tumor-derived neo- and self-antigens via dermal DCs. These responses suppressed the growth of cutaneous tumors and disseminated melanoma lacking the Trm cell-targeted epitope. Moreover, analysis of RNA sequencing data from human melanoma tumors reveals that enrichment of a Trm cell gene signature associates with DC activation and improved survival. This work unveils the ability of Trm cells to increase the breath of cytotoxic CD8+ T cell responses through DCs to strengthen anti-tumor immunity

Keywords: Resident memory CD8+ T cells, Dendritic cells, Antigen spread.

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Vaccination-induced skin-resident memory CD8+ T cells mediate strong protection against cutaneous melanoma. Gálvez-Cancino F, López E, Menares E, Díaz X, Flores C, Cáceres P, Hidalgo S, Chovar O, Alcántara-Hernández M, Borgna V, Varas-Godoy M, Salazar-Onfray F, Idoyaga J, Lladser A. Oncoimmunology. 2018 Mar 19;7(7):e1442163. doi: 10.1080/2162402X.2018.1442163

## A208 / CD4+ T-cells induce rejection of urothelial tumors after immune checkpoint blockade

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Immune checkpoint blockade (ICB) provides clinical benefit to a minority of patients with urothelial carcinoma (UC). The role of CD4+ T-cells in ICB-induced anti-tumor activity is not well defined, but they are speculated to play a supportive role to CD8+ T-cells that kill tumor cells after recognition of tumor antigens presented by MHC class I. To investigate the mechanisms of ICB-induced activity against UC, we developed mouse organoid-based transplantable models that have histologic and genetic similarity to human bladder cancer. We found ICB can induce tumor rejection and protective immunity with these systems in a manner dependent on CD4+ T-cells, but not reliant on CD8+ T-cells. Evaluation of tumor infiltrates and draining lymph node after ICB revealed expansion of IFNγ producing CD4+ T-cells. Tumor cells in this system express MHC class I, MHC class II, and the IFNγ receptor (Ifngr1), but none were necessary for ICB-induced tumor rejection. IFNγ neutralization blocked ICB activity, and in mice depleted of CD4+ T-cells, IFNγ ectopically expressed in the tumor microenvironment was sufficient to inhibit growth of tumors in which the epithelial compartment lacked Ifngr1. Our findings suggest unappreciated CD4+ T-cell dependent mechanisms of ICB activity, principally mediated through IFNγ effects on the microenvironment.

Keywords: CD4+ T cell, PD-1, CTLA-4, IFNγ.

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## A209 / Modular architecture of the STING C-terminal tail allows interferon and NF-κB signaling adaptation

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Stimulator of Interferon Genes (STING) is a key regulator of type I interferon and pro-inflammatory responses during infection, cellular stress, and cancer. Here we reveal a mechanism for how STING balances activation of IRF3- and NF-κB-dependent transcription, and discover that acquisition of discrete signaling modules in the vertebrate STING C-terminal tail (CTT) shapes downstream immunity. As a defining example, we identify a motif appended to the CTT of zebrafish STING that inverts the typical vertebrate signaling response and results in dramatic NF-κB activation and weak IRF3-interferon signaling. We determine a co-crystal structure that explains how this CTT sequence recruits TRAF6 as a new binding partner, and demonstrate that the minimal motif is sufficient to reprogram human STING and immune activation in macrophage cells. Together, our results define the STING CTT as a linear signaling hub that can acquire modular motifs to readily adapt downstream immunity.

Keywords: STING, NF-κB, innate immunity, TRAF6.

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de Oliveira Mann, C. C., Orzalli, M. H., King, D. S., Kagan, J. C., Lee, A. S., & Kranzusch, P. J. (2019). Modular architecture of the STING C-terminal tail allows interferon and NF-κB signaling adaptation. Cell reports, 27(4), 1165-1175.

## A210 / Role of Dectin-1 and Nitric-Oxide Synthase type 2 in NOD.RET+ transgenic mice, a new mouse model of rapidly evolving melanoma. Implications for human cancers

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Mice transgenic for the RET oncogene provide a remarkable model for investigating the mechanisms underlying the promotion and the development of melanoma tumors. In this model, as in human melanoma, vitiligo is associated with improved prognosis. We inferred that RET-transgenic mice with the autoimmune-prone non-obese diabetic (NOD) genetic Background should be protected

against melanoma development.

We bred (NODxB6) F1 mice and backcrossed them with NOD mice. Very unexpectedly, F1 mice and mice at subsequent generations of backcrossing showed marked acceleration of tumor development, in particular with a more frequent and an earlier extension of the tumors from the primary ocular site. We then investigated whether changes in the immune system could explain this accelerated evolution of tumors. In close relation with this severe tumor evolution, we observed a profound drop in Dectin-1 expression on granulocytic myeloid cells CD11b+Ly6G+ correlating with an upregulation of the CD4+Foxp3+ T regulatory cell subset and of the CD8+IFNγ+ T cell population. Interferon (IFN)-γ is a major inducer of the type 2 nitric-oxide synthase (Nos2) gene whose NO products are known to be tumorigenic. Germline inactivation of the Nos2 gene was associated with a dramatically improved survival tumor prognosis and a restoration of Dectin-1 expression on myeloid cells. Moreover, in vivo treatment of (NODxB6) F1.RET+ mice with curdlan, a glucose polymer that binds Dectin-1, prevented tumor extension and was associated with marked reduction of the CD4+Foxp3+ T cell subset. In parallel, we studied the prognostic value of NOS2 expression in four type of epithelial human cancers, including melanoma, nasopharyngeal, colorectal and breast tumors. Overall, we observed that an increased expression of NOS2 was associated with more severe clinical and histopathological characteristics. These observations highlight the NOD strain as a new model to investigate the role of the immune system in the host-tumor relationship and point to Dectin-1 and Nos2 as potentially promising therapeutic targets, especially in light of clinical findings.

Keywords: Dectin-1, Nos2, Myeloid cells, Melanoma.

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## A211 / Overcoming resistance to immune checkpoint inhibitors by targeting TREM2+ tumor-associated macrophages

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One of the major challenges in the immuno-oncology field is identifying therapies that convert immune checkpoint inhibitor (CPI)-resistant patients into therapy-sensitive patients. The basis for CPI resistance involves multiple mechanisms, one of which includes the role of tumor-associated macrophages (TAMs). TAMs are thought to be one of the major sources of innate CPI resistance as they employ a multitude of means to subvert anti-tumor immunity and directly promote tumor growth. Clinically, high levels of TAMs predict poor patient prognosis across multiple solid tumor indications. Consequently, therapeutic targeting of TAMs by compromising their survival and/or modulating TAM function is a promising strategy to both augment response rates in solid tumor indications and overcome resistance to CPI therapies. To this end, we and others have identified the transmembrane protein TREM2 (Triggering Receptor Expressed on Myeloid cells 2) as a highly enriched TAM marker. To target TAMs, we developed an anti-TREM2 monoclonal antibody (mAb), termed PY314m, that demonstrated compelling anti-tumor activity in combination with an anti-PD-1 mAb in a number of preclinical, anti-PD-1 resistant mouse syngeneic tumor models. Furthermore, PY314m also exhibited strong single-agent activity in a subset of these tumor models. Mechanistically, PY314m significantly reduced a subset of TAMs, namely the M2-like, MHCII-Low, and ArginaseHigh TAMs, expanded MHCII-High M1-like TAMs, and was associated with the release of pro-inflammatory cytokines. An important advantage of depleting M2-like TAMs, as opposed to targeting just a single TAM-mediated immune-suppressive pathway, is that PY314m treatment removes multiple immune-suppressive mechanisms. PY314m treatment also resulted in an increase in the absolute number of intra-tumor immune cells that are known to drive anti-tumor responses, including cytotoxic CD8+ T cells. PY314m plus anti-PD-1 mAb combination treatment produced long-term immunological memory as evidenced by the lack of tumor growth upon rechallenge in mice cured of their tumors. Screening of human tumor microarrays for TREM2 expression indicated that TREM2+ TAMs were expressed in multiple human solid tumors. Collectively these findings suggest that anti-TREM2 mAb therapy could be used to overcome CPI resistance in humans. To this end, we developed a humanized version of PY314m termed PY314, that in the pilot, repeat- dose non-GLP studies in non-human primates was well tolerated and had good pharmaceutical properties. Our ongoing efforts are aimed at better defining CPI-resistant patient populations that will maximally benefit from PY314 therapy.

Keywords: Tumor-associated macrophages, anti-tumor immunity, checkpoint inhibitor resistance, combination immunotherapy.

## A212 / Post-translationally modified antigens are good targets for cancer immunotherapy but some patients have antigen specific T-regs that may need to be neutralized

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The object of this study was to evaluate the immunogenicity of citrullinated epitopes in cancer. Stress induced post-translational modifications (siPTM) stimulate T cell responses that result in anti-tumor immunity. Citrullination is the post-translational modification of arginine to citrulline by peptidyl deiminase (PAD) enzymes which are activated by high levels of calcium. In viable cells high levels of calcium can only occur in double membrane organelles such as nuclei and autophagosomes. Carbamylation of lysine by chemical reaction of isocyanic acid leads to homocitrullination. Myeloperoxidase (MPO) produced by myeloid suppressor cells catalyzes the build-up of isocyanic acid which diffuses into the tumor cells and homocitrullinates cytoplasmic proteins. Modified proteins are then degraded and presented by MHC-II. In murine tumor models, immunization with citrullinated or homocitrullinated epitopes resulted in strong anti-tumor immunity and long-term survival (1-4). Here we show that cancer patients have a repertoire to these siPTM peptides when their PBMCs (peripheral blood mononuclear cells) are cultured and stimulated with our citrullinated/homocitrullinated peptides. A total of 37 cancer patients (16 lung cancer, 16 ovarian cancer and 5 breast cancer) and 30 healthy donors were bled. PBMCs were isolated, CD25 depleted, CFSE loaded then cultured with one of the citrullinated or homocitrullinated peptides. 77% of healthy donors, 56% of lung cancer patients, 63% of ovarian cancer patients and 60% of breast cancer patients showed a specific CD4+ proliferative response to at least 1 of the 9 citrullinated/homocitrullinated peptides. Cytokine expression in early activated CD71+ cells was also measured in breast and lung patients. Healthy donors, lung cancer and breast cancer patients showed IFNγ responses but antigen specific CD4+CD71+ cells and CD4+CFSElo were significantly more positive for LAP in cancer patients compared to that seen in the same population in healthy donors ($p = 0.0001$, $p = 0.0109$). In all donors, there is also an inverse correlation between CD4+CD71+LAP+ cells and proliferation (CD4+CFSElo) (p value 0.001). Skurr et al. (5), has described this population of CD4+ LAP+ cells as being 50-fold more suppressive than the conventional CD4+CD25+Foxp3+. These results suggest that modified antigens may be a good target for anti-tumor immunity but that some cancer patients may have developed an antigen specific LAP+ regulatory population which would either need to be inhibited with a TGF-β neutralizing antibody or polarized to Th1 with vaccination.

Keywords: citrullination, LAP, CD4+ T-cells, Cancer.

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## A213 / Novel therapeutic approach to upregulate immunostimulatory targets for cancer therapy

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Small activating RNAs (saRNAs) are short double-stranded oligonucleotides designed to upregulate target gene expression by transcriptional activation. saRNAs transfected into cells are loaded into Ago2 and translocate into the nucleus where they interact specifically at the target gene leading to recruitment and activation of RNA Polymerase II (Portnoy et al., 2011). This leads to new mRNA production resulting in upregulation of the target protein. We have developed a saRNA based therapeutic, MTL-CEBPA to target the expression of the CEBPA transcription factor. MTL-CEBPA has been shown to inhibit hepatocellular cancer tumor growth in preclinical models and to enhance the activity of immune checkpoint inhibitor drugs through impact on the tumor microenvironment (Reebye et al., 2018). This is the first saRNA drug to enter clinical trials and we are investigating whether this technology could be applied to activate immune-stimulatory genes for utilization in cancer immunotherapy.

Using our proprietary algorithm we design saRNA sequences that bind to long non-coding RNA +2000 to -2000 nucleotides upstream or downstream from the transcriptional start site. saRNAs were designed and synthesized to target the IL23A, IL36g and OX40L transcripts. Double-stranded 19-nucleotide sequences from the algorithm for each target were added with mUmU overhangs at the 3' ends of both strands to improve stability and reduce non-specific immunogenicity. The saRNAs were screened in human (A549 and HepG2) or murine (CT26, BNL.1ME and RAW264.7) cells by transfection and mRNA levels of the target genes were measured by qPCR.

The saRNA algorithm identified active saRNAs for all 3 targets leading to upregulation of target mRNA at 48 -72 hours post-transfection compared to oligonucleotide transfection controls. The levels of mRNA upregulation for each leading saRNA sequence from this screen were: IL23A (4.9 fold), IL36g (3.5 fold) and OX40L (6.7 fold). Sequence-dependent specificity of the lead saRNA were confirmed by switching the positions of 2 to 3 nucleotide residues in the seed portion of the sequences (nucleotides 2-8 in the anti-sense strand) which reduced induction of target mRNA compared to the parental saRNA sequence. The presence of increased target protein in saRNA transfected cells was confirmed either by immunofluorescence or ELISA. The heterodimeric IL23 cytokine is composed of the IL23A and IL12B subunit. We found that saRNA targeting IL23A expression increases IL12B expression indicating an increase of functional IL23. Furthermore, we observed that co-transfection of cells with a combination of IL23A and IL36g saRNA improved IL36g expression (from 3.5-fold with single IL36g saRNA to 13.1-fold with a combination of IL23A and IL36g saRNA). The induction of IL12B mRNA was also maintained using the combination of IL23A and IL36g saRNA.

In summary, we have demonstrated the ability of saRNA technology to upregulate immune-stimulatory targets for utilization in immunotherapy. We are currently optimizing formulation for delivery of the lead saRNAs for efficacy studies in preclinical tumor models.

Keywords: small activating RNA, transcriptional activation, immune stimulatory genes.

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## A214 / Reporter imaging of Trp-IDO/TDO-Kyn-AHR pathway activity

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Background: Despite recent progress in immunotherapy (checkpoint blockade and adoptive T cell transfer), most patients with solid tumors still do not respond or develop resistance to therapy. Our group and others have described an immune resistance mechanism mediated by the metabolic dysregulation of Tryptophan (Trp) catabolism (mediated by indoleamine-2, 3-dioxygenases (IDO) and 2, 3-dioxygenase (TDO)) to Kynurenine (Kyn) and the aryl hydrocarbon receptor (AHR). The production of Kyn and signaling through the AHR pathway, suppresses CD8+ and CD4+ effector T cells and enhances the generation of immunosuppressive cell types, including FoxP3+CD4+ T cells (Tregs), myeloid-derived suppressor cells (MDSCs) and M2-polarized tumor-associated macrophages (TAMs). These cells play a critical role in limiting anti-tumor immunity. In order to optimize the scheduling of combination drug treatment (treatments targeting this pathway combined with immune based therapies), we developed a reporter system to image signaling activity through the IDO/TDO-Kyn-AHR pathway.

Methods: A first generation Kyn/AHR-sensitive luciferase reporter, driven by a dioxin response element (DRE), was developed to image Kyn/AHR signaling in B16 melanoma cells and 4T1 breast cancer cells (wild-type and HSV1-tk-GFP-FLuc fusion) using bioluminescence imaging (BLI). Since IDO over-expressing B16 melanoma cells are in the process of being transduced with the Kyn/AHR reporter (DRE), we performed "co-injection" (IDO-B16 plus DRE-B16) experiments, where IDO-B16 (non-reporter) cells were mixed with DRE-B16 (reporter) cells, and implanted s.c. for imaging AHR activation and suppression. Similar tumor imaging studies were performed with AHR suppression in the s.c. the DRE-4T1 tumor model. results. Two AHR activating molecules (kynurenine and the AHR agonist FICZ) were observed to increase luciferase expression, in B16-DRE and 4T1-DRE reporter cells, whereas an AHR inhibitor (CH223191) reduced reporter activity. Similarly, in B16 melanoma animal models where cells with over-expressed IDO were combined and co-injected with B16 DRE reporter cells, the resultant tumors showed increased tumor growth and increased reporter activity (BLI signal). These reporter imaging

Results: were consistent with the significant differences observed between B16-IDO and B16-wt tumors: i) greater immune cell infiltration, ii) greater activation of T regs and TAMs, and iii) greater expansion of suppressive myeloid cells were observed in B16-IDO tumors. LDH-A depletion in 4T1 tumors significantly reduced tumor tryptophan and increased kynurenine levels, and was associated with a significantly higher density of CD3+ T cells.

Comment:. An inducible DRE-responsive reporter system was developed to better understand signaling through the Trp–Kyn-AHR pathway in the tumor microenvironment. Coupled with a constitutive reporter in the same cells, it is possible to quantitatively monitor AHR transcriptional activity using this dual reporter system. The dual reporter system is expected to: i) quantify the kinetics of engagement with the AHR upon treatment with different agonists/antagonists and monitor the correlation with phenotypic changes in different components of the TME, including cancer cells, macrophage and T cells; ii) monitor the dynamic activation of the AHR pathway in vivo during tumor progression and targeted treatment in IDO/TDO-expressing cancer models;

iv) evaluate the in vivo dynamics of AHR activation after response to immune-based therapeutic interventions (PD-1/CTLA-4 blockade, T cell therapy) in the same models; v) evaluate the potential for clinical translation using radionuclide-based human reporter genes.

Keywords: Trp-IDO/TDO-Kyn-AHR pathway, reporter imaging, imaging AHR pathway activity, dual (inducible and constitutive) reporter imaging.

## A215 / Tissue-specific innate lymphoid cells are novel targets for pancreatic cancer immunotherapy

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Pancreatic ductal adenocarcinoma (PDAC) is the classic cold tumor, with few tumor-infiltrating activated CD8+ T cells, a major barrier to immunotherapy. Strategies to convert cold tumors like PDACs to hot can enable more effective immunotherapies. To identify targets that can convert cold PDACs to hot, we compared a large cohort of rare long-term PDAC survivors with hot tumors to short-term survivors with cold tumors. Surprisingly, hot PDACs were enriched not only in activated CD8+ T cells, but also in group-2 innate lymphoid cells (ILC2s), a class of largely tissue-resident, cytokine-producing innate lymphocyte that potentiate T cell immunity in local tissues. We identified that tumor ILC2s (TILC2s) infiltrate human and mouse PDACs, and TILC2 frequency and tumor expression of the ILC2-activating ligand interleukin (IL)-33 positively correlate with tumor immune cytolytic activity, and long-term patient survival. Using PDAC mouse models, we discovered that the IL33-ILC2 axis activates pancreatic tissue-specific anti-tumor T cell immunity. Host IL33 deficiency attenuated TILC2 expansion and CD8+ T cell activation, accelerating tumor growth in orthotopic PDACs but not heterotopic skin implanted PDACs where TILC2s lack the IL33 receptor. Acute ILC2 depletion partially phenocopied the tissue-specific phenotype of IL33 deficiency, with impaired rejection of immunogenic orthotopic but not heterotopic skin PDACs. Strikingly, chronic treatment with recombinant IL33 (rIL33), but not with the ILC1-activating ligand rIL18, increased TILC2 frequencies by 10-fold, activated CD8+ T cells by 5-fold, and abrogated PDAC establishment in >70% of mice in a pancreatic tissue-specific, ILC2-dependent manner. Taken together, these studies identify ILC2s as novel anti-cancer immune cells for PDAC immunotherapy. Strategies to activate tissue-specific ILC2s may be a promising immunotherapeutic approach for PDAC and other malignancies with similar immune landscape.

Keywords: Innate lymphoid cells, IL33, Pancreatic Cancer.

## A216 / Macrophage phenotype plasticity regulates radiation-induced lymphocyte migration

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Radiotherapy can increase leukocyte infiltration into tumors and enhance anti-tumor immunity in preclinical models (1). Macrophages are tissue resident regulators of leukocyte infiltration, which show phenotype plasticity on a continuum between anti-tumor and pro-tumor function (2). Here, we investigated the effects of ionizing radiation (IR) on macrophage tumor cell interactions and subsequent effects on leukocyte migration using an in vitro coculture system. Human monocyte-derived macrophages were cocultured with human pancreatic adenocarcinoma cell lines and irradiated using a 137Cs source. Macrophage polarization was assessed by flow cytometric (FACS) analysis of selected surface proteins and cytokine expression was determined by quantitative reverse transcription polymerase chain reaction (qRT-PCR) of RNA isolated from macrophages sorted by FACS. Leukocyte migration towards cocultures was determined using modified Boyden chamber assays and chemokine expression was assessed using flow cytometry-based multiplex bead-arrays and qRT-PCR of FACS-sorted macrophages. In the coculture system, macrophages acquired an immunosuppressive phenotype, which was attenuated by radiation in a dose dependent manner. IR had no effect on macrophage polarization in monoculture, indicating that macrophage-tumor cell interactions were pivotal for the observed radiation-induced changes in macrophage polarization. This shift in macrophage polarization was accompanied by increased CD4+ T cell, CD8+ T cell, NKT cell, and CD56dim NK cell migration and changes in chemokine expression patterns in the irradiated cocultured macrophages, both on protein and transcript level. This study thereby expands our mechanistic understanding of macrophage-tumor cell interactions post-radiation, which may be exploited to improve current radio-immune combination therapies.

Keywords: Radiation, Chemotaxis, innate immunity, myeloid.

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## A217 / Synthetic gene circuits for cancer immunotherapy: Turning cancer cells against themselves.

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Cancer immunotherapy has demonstrated robust therapeutic efficacy in clinics, but challenges such as the lack of ideal targetable tumor antigens, severe systemic toxicity, and tumor-mediated immunosuppression still limit its success. To overcome these challenges, we have designed a synthetic cancer-targeting gene circuit platform that triggers a robust tumor-localized combinatorial immunotherapy from within cancer cells: a Trojan horse-like approach. Once the circuits are introduced into cells, they will sense and integrate two pre-defined cancer-specific transcription factor activities with a logical AND gate. Only when both transcription factor activities are high, the circuit will trigger an effective combinatorial immunotherapy selectively from within cancer cells, while keeping normal cells unharmed. The circuit demonstrated robust therapeutic efficacy in treating disseminated ovarian cancer in mouse models. This platform can be adapted to treat multiple cancer types and can potentially trigger any genetically-encodable immunomodulators as therapeutic outputs. Moreover, this gene circuit platform can be adapted to treat additional diseases exhibiting aberrant transcription factor activities, such as chronic metabolic diseases and autoimmune disorders.

Keywords: synthetic biology, cancer-targeting gene circuit, cancer immunotherapy, combination immunotherapy.

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## A218 / Transcriptomic profiling of cytotoxic and regulatory T cells in acute myeloid leukemia identifies novel potential drug targets

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Acute myeloid leukaemia (AML) has a 5-year survival of <5% among patients over 65 years. Checkpoint inhibitors have shown extraordinary effects in certain cancer patient groups, however, the results have been modest in AML. The aim of this project is to identify novel immunotherapeutic drug targets for treatment of AML based on transcriptomic analysis of cytotoxic T cells (CTLs) and regulatory T cells (Tregs).

Flow cytometry was used to sort out CTL and Treg populations from peripheral blood of AML patients with TP53 mutations (n=5), as well as control subjects (n=3). RNA was extracted and was subjected to RNA sequencing. Data was analysed using Qlucore Omics Explorer software and MetaCore. To study the impact of AML on T cells, transcriptome of each T-cell population in the AML cohort was compared against its counterpart in the control cohort and differentially expressed genes were identified (two-tailed t-test, FDR<0.1). Additionally, two strategies were used to identify novel drug target candidates. Firstly, genes expressed higher in CTLs in AML as compared to CTLs in control cohort were identified (one-tailed t-tests, FDR<0.1), and similarly for Tregs. In the second strategy, targets for selective manipulation of CTLs and Tregs in AML patients were identified based on the genes expressed higher in CTLs in AML as compared to Tregs in AML and vice versa (one-tailed t-test, FDR<0.1). For both strategies, genes associated with plasma membrane (i.e. available to targeted therapy) were subsequently extracted and those that were novel in the context of immunotherapy in AML were identified based on literature.

The analysis identified 578/498 and 391/387 genes expressed at higher/lower level by CTLs and Tregs from AML compared to their peer populations in control subjects, respectively. Interestingly, 22 receptor-encoding genes were expressed higher in CTLs from AML patients as compared to the same population in control subjects, including 15 potential drug targets. For Tregs, 17 receptor-encoding genes were expressed at higher levels in AML patients as compared to its peer population in control cohort, including 9 potential drug targets. Furthermore, 30 plasma membrane-associated genes were expressed selectively by CTLs compared to Tregs in AML patients, including 6 potential drug targets. Also, 5 plasma membrane-associated genes were found to be expressed selectively by Tregs compared to CTLs in AML patients, including 2 potential drug targets. To our knowledge, 7 of the identified potential drug targets are novel and have not been investigated previously within the context of AML immunotherapy.

In summary, gene expression profile of CTLs and Tregs in AML is different from that of the counterpart population in control subjects. The identified differentially expressed genes can give mechanistic insights into the impact of AML on T cells, and were used to pinpoint several potential novel drug targets in AML. Additionally, drug target candidates for specific stimulation of CTLs or inhibition of Tregs in AML patients were identified.

Keywords: Acute myeloid leukemia, Cytotoxic and regulatory T cells, RNA sequencing, Immunotherapy.

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## A219 / Targeting urokinase-type plasminogen activator receptor (uPAR) with an HLA-independent αβ T-cell receptor

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The GPI-anchored urokinase-type plasminogen activator receptor (uPAR; aliases: PLAUR, CD87) is considered as a relevant diagnostic and therapeutic target on cancer cells. uPAR and its ligand urokinase (uPA) are involved in regulating tumor invasion and metastasis, cancer cell survival and angiogenesis. Enhanced expression of the uPA complex has been observed in a variety of common solid tumors and hematological malignancies and has been associated with a worse prognosis. E.g., uPAR radioligands are currently investigated in PET imaging trials. Previously, we have observed the emergence of an HLA-independent anti-tumor αβT-cell repertoire in melanoma patients with HLA I-negative disease. This finding prompted us to see, if uPAR can be targeted by HLA-independent T cells. We stimulated CD3+ umbilical cord blood (UCB) lymphocytes with a genetically engineered HLA I/II-deficient melanoma cell line overexpressing uPAR and stably transfected with CD80 and CD83. Responder T cells were analyzed for their specificity primarily with HEK293T cells also genetically modified for HLA deficiency and either lacking uPAR expression or transfected with uPAR. In 2/2 UCB donations we readily detected T cells recognizing both stimulators and uPAR-transfected readout target cells. Their specificity for uPAR was additionally confirmed by complete inhibition with an anti-uPAR monoclonal antibody. An αβTCR was cloned from uPAR-specific T cells, chimerized with murine constant domains, codon-optimized and cloned into a P2A-based bicistronic retroviral construct. The TCR construct was transduced into blood T cells of a healthy donor. Both CD8+ and CD4+ T cells expressing the TCR specifically recognized uPAR in an HLA-independent, but TCR-dependent manner both in lysis and ELISPOT assays. Notably, this TCR cross-reacted with the murine uPAR homologue. TCR-transduced T cells recognized all tested primary AML blast populations (FAB M4/M5, obtained from 19 patients). However, although uPAR is known to be expressed in normal hematopoietic cells, TCR-transduced T cells recognized neither monocytes nor immature or mature dendritic cells. We currently investigate the precise molecular mechanism of uPAR recognition. So far, we have found that HLA-independent T-cell recognition of uPAR is abolished after deletion of the protein´s GPI anchor, depends on the protein´s glycosylation and requires the presence of the physiological trimolecular complex consisting of uPAR with its intact three-domain structure, uPA and vitronectin (VN). But it is still

unclear, which region of the uPAR/uPA/VN complex is directly involved in the binding of uPAR-reactive TCR and in which way this complex differs e.g. in AML cells as compared with non-malignant hematopoietic cells. The therapeutic use of such an HLA-independent TCR would counteract numerous escape mechanisms affecting HLA/peptide-specific T cells and also allow to circumvent the dictate of HLA polymorphism.

Keywords: uPAR, T cell receptor.

## A220 / Targeting nodal and cripto-1 onco-fetal proteins using bispecific antibody fragments

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Targeted therapies using monoclonal antibodies and their combination regimens are considered as 'Magic Bullets' for treating cancer. The increasing understanding of the cancer-immune system relationships and the advancements in protein engineering technology together have paved the way to the design and development of ever more specific biomolecules. These efforts have ultimately led to introducing the concept of bispecific (or even multispecific) antibodies grasped as single agents with dual or multi-targeting features with high potential as novel therapeutics to fight cancer. We are generating bispecific fabs hunting dual epitopes or dual targets in order to improve specificity and efficacy toward diseased cells or tissues. Here, our targets of interest are two onco-fetal proteins Nodal and Cripto-1. Nodal is a potent embryonic morphogen belonging to the TGF-beta superfamily. Typically, it binds to the Alk4/ActRIIB receptor complex in the presence of the co-receptor Cripto-1. Nodal and Cripto-1 expression is restricted to embryonic tissues and human embryonic stem cells, whereas it is poorly present in normal adult cells. Re-expression of these proteins in the adults are associated with many tumors where they can control intracellular signaling and promote tumorigenesis. The two proteins have been thereby indicated as diagnostic biomarkers and therapeutic targets for several types of cancer. We have generated anti-Nodal and anti-Cripto-1 monoclonal antibodies named 3D1 and 1B4, respectively. 3D1 therapeutic efficacy has been proven in aggressive melanoma both in vitro and in vivo models. Beyond to block Nodal and Cripto-1 cellular signaling separately, 1B4 and 3D1 mAbs also may take advantage of working in combination acting as potential double neutralizing molecules that block the whole Nodal/ Cripto-1/Alk4 ternary complex. To investigate this hypothesis at the therapeutic level, we have generated partly humanized recombinant Fabs of 3D1 and 1B4 to obtain new molecules with better PK/PD profiles. We obtained the 3D1 recombinant chimeric Fab and recombinant chimeric Fab of 1B4 by expressing in the E. Coli host using the periplasmic expression strategy. These chimeric antibody fragments will be employed as the fragments to generate the corresponding bispecific anti-Cripto-1/anti-Nodal Fab2 for both imaging and therapeutic purposes in Cripto-1/Nodal positive tumors. To selectively obtain the bispecific molecule we have applied a recently developed conjugation strategy that makes use of short peptide-based linkers introduced on the C-terminus of the Fab's heavy chains exploiting MTGase transglutamination (bio-conjugation) reaction. The presence of specific orthogonal chemical groups ensures site-specific heterodimerization that leads only to bispecific molecules. We present the biochemical characterization of the 3D1 and 1B4 recombinant chimeric Fab efficiently produced in the E. Coli host using the periplasmic expression strategy. Expression and purification conditions have been optimized. Biochemical characterizations have confirmed the folding (Circular Dichroism), identity (ESI TOF mass spec), evaluated the kinetic binding and functionalization of these recombinant chimeric fabs (SPR, BLItz, ELISA, Western blotting) of anti-nodal 3D1 fab and anti-cripto-1 1B4 fab against its target antigens. Furthermore, using short linkers we have introduced fluorescent dyes and the resulting molecules may have a direct use for imaging applications. This optimized Fab-FITC conjugation reaction confirms the yield of more than 90% and its purity and identity are confirmed by ESI TOF Mass spectrometry. Using the same concept, the Fab of anti-Nodal 3D1 and the Fab of anti-Cripto-1 1B4 molecules will be dimerized to generate the bispecific antibody Fab2 that will be finally employed as the dual anti-Nodal and anti-Cripto-1 theranostic agent.

Keywords: Bispecific, Antibody fragments, Nodal, Cripto-1.

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## A221 / Losartan treatment lowers solid stress in ovarian cancer

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Solid stress, distinct from fluid pressure, is a physical force contained in and transmitted by solid components of the tumor including cells and the matrix they produce. Tumor blood and lymphatic vessels are highly structurally abnormal and are easily collapsible under this mechanical force, resulting in reduced blood and lymphatic flow throughout the tumor mass. This leads to (i) decreased drug delivery to tumors, which compromises treatment efficacy, (ii) lymph accumulation, and (iii) increased tumor hypoxia, which promotes aggressive phenotypes, immunosuppression, and resistance to therapies that require oxygen to be effective. Losartan, a common antihypertensive that can reduce tumor extracellular matrix, is known to decrease solid stress. To that effect, we investigated the effects of losartan on solid stress in mouse models of orthotopic ovarian tumors.

Ovarian tumors (SKOV3ip1) from either control or losartan-treated mice were excised and subjected to solid stress measurements. Briefly, tumors are sliced in half to release the mechanical forces that make up solid stress, which causes the tissue to deform in peaks (areas originally under compression) and valleys (areas originally under tension). The tissue deformation is imaged via high-resolution ultrasound, and post-processed via commercially available software to produce a 2-D map of the resultant solid stress.

In size-matched peritoneal tumors, losartan treatment led to significant reduction in compressive stresses by 25.5%, and tensile stress by 29.8%. Histological and imaging studies confirmed that losartan treatment, via its antifibrotic effects, is capable of decompressing vessels by reducing solid stress exerted from the dense tumor ECM. Indeed, losartan enhances the delivery and efficacy of chemotherapeutics in pancreatic, breast and ovarian cancer in mice and patients, and has the potential to do so for immunotherapies. Furthermore, solid stress measurements provide a new class of mechanical biomarkers that can be correlated to clinical outcomes for predictive/prognostic value.

Keywords: Reprogramming Tumor Microenvironment, Novel Therapeutics, Drug Delivery, Biomarker.

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## A222 / HLA open conformers induces potent anti-tumor activity by blocking LILRB and KIR receptors and acts synergistically with check-point blockade inhibition

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Introduction: HLA open conformers (OC) are defined as HLA class I molecules lacking beta-2-microglobulin (β2m) and peptide. OC can be derived from different HLA I molecules such as HLA-B27, -B57, and –Cw08. OC have a different three dimensional structure when compared to their respective HLA/β2m/peptide counterpart and induce distinct immunological processes by binding to LILRB and KIR molecules, both key receptors of the innate immune system. B57 OC expression is associated with enhanced immunity against viruses and can cause autoimmunity. Its potential anti-tumor activity has not been exploited so far.

Methods: B57 OC and control molecules were expressed as Fc-fusion proteins in CHO cells. The affinity for protein-ligand interaction was measured by SPR. Human macrophages M1/M2, polarization, phagocytosis, NK cell & CD8+ T cell cytotoxicity were assessed by flow cytometry and cellular assays. Syngeneic pancreatic cancer (Pan02), colon cancer (MC38) mouse models and melanoma transgenic mice were used. Mice with tumors of 80mm3 were treated twice weekly at 5 mg/kg. Tumor and blood samples were analysed. FDA cancer panels were assessed by IHC for LILRB1-5 expression. Results: B57 OC displays distinct protein-ligand interactions with high affinity binding to LILRB2 & 4, KIR2L1-3, and KIR3DL1. Therapeutic efficacy in pancreatic and colon cancer models was observed with monotherapy ($p<0.01$), and combo therapy using PD-1 and/or 41BB antibodies ($p<0.0001$). Significant tumor reduction and survival efficacy was observed in transgenic melanoma mice. Ex vivo tumor sample analysis revealed a significant reduction of MDSC & Tregs, and an increase of M1 type macrophages. In addition, loss of MDSC functionality and enhanced CD8+ T cell expansion and cytotoxicity was assessed. IHC of human tissue demonstrated enhanced LILRB2 & 4 expression, notably in colon and lung cancer.

Conclusion: B57 OC has a unique binding profile to LILRB and KIR receptors. B57 OC induces anti-tumor activity in diverse syngeneic and transgenic mouse models and acts synergistically with PD1 or 41BB antibodies. Pre-clinical and human in vitro experiments demonstrate that B57 OC directly augments anti-tumor immunity through inhibition of MDSCs, M1 polarization of macrophages, activation of NK cells and LILRB blockade on tumor cells, which, in turn, indirectly boosts adaptive immune system by increasing CD8+ effector T cells and reducing Treg numbers. B57 OC is a first-in-class therapeutic with robust anti-tumor activity.

Keywords: HLA open conformers, Immuno-oncology.

# Onco-and immunometabolism

## A223 / Tumor lysis with LTX-401 creates anticancer immunity

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Local immunotherapies such as the intratumoral injection of oncolytic compounds aim at reinstating and enhancing systemic anticancer immune responses. LTX-315 is a first-in-class, clinically evaluated oncolytic peptide-based local immunotherapy that meets these criteria. Here, we show that LTX-401, yet another oncolytic compound designed for local immunotherapy, depicts a similar safety profile and that sequential local inoculation of LTX-401 was able to cure immunocompetent host from subcutaneous MCA205 and TC-1 cancers. Cured animals exhibited long-term immune memory effects that rendered them resistant to rechallenge with syngeneic tumors. Nevertheless, the local treatment with LTX-401 alone had only limited abscopal effects on secondary contralateral lesions. Anticancer effects resulting from single as well as sequential injections of LTX-401 were boosted in combination with PD-1 and CTLA-4 immune checkpoint blockade (ICB), and sequential LTX-401 treatment combined with double ICB exhibited strong abscopal antineoplastic effects on contralateral tumors underlining the potency of this combination therapy.

Keywords: Oncolysis, anticancer therapy, immunogenic cell death, checkpoint blockade.

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## A224 / Oxidative metabolism promotes apoptotic resistance in Th17 cells

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A limitation to long-term efficacy of T cell-based immunotherapy is T cell survival. Following activation, T cells are programmed to undergo apoptotic cell death to limit unnecessary inflammation, and those that escape apoptosis persist to provide long-term immunity. Cellular metabolism fuels T cell homeostasis and growth. Moreover, given the role of metabolism in the expansion and effector phases of an immune response, it is of importance for anti-tumor immunity to understand how the metabolic needs of effector T cells dictate their potential for survival or death. Indeed, short-lived effector T cells utilize glycolysis for rapid expansion, while T cells that rely on mitochondrial oxidative phosphorylation (OXPHOS) are suggested to be metabolically equipped for memory cell persistence and function. These bioenergetic preferences suggest a metabolic checkpoint that controls T cell fate and thus, the degree of an immune response. Th17 cells represent a subset of CD4 T cells that have an important role in anti-tumor immunity. We have previously shown that Th17 cells in vivo require OXPHOS during effector phases and under chronic inflammatory conditions. This in vivo metabolic requirement is in contrast to in vitro-derived Th17 cells which are predominately glycolytic. To better understand how OXPHOS influences Th17 cell fate and function, we developed in vitro culture conditions that generate Th17 cells that metabolically resemble their in vivo counterparts. Using this system, we have found that Th17 cells can use either glycolysis or OXPHOS for proliferation, differentiation and cytokine production. However, compared to their glycolytic counterparts, OXPHOS Th17 cells are more resistant to apoptotic stimuli in vitro and exhibit improved survival and persistence in vivo. Mechanistically, we have revealed that the reliance on OXPHOS vs. glycolysis leads to altered expression of BCL-2 anti- vs. pro-apoptotic regulators. Our work provides new insight into the role of OXPHOS in Th17 cell fate and may lead to the development of novel approaches that aim to inhibit or amplify Th17 responses.

Keywords: Th17 cells, Apoptosis, immunometabolism.

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## A225 / Reprograming of innate immunity by glioma-derived R-2-Hydroxyglutarate

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Primary low-grade gliomas (LGG) represent a group of diffusely infiltrating brain tumors that often affect young adults and eventually transform to higher grade gliomas including glioblastoma. Isocitrate dehydrogenase 1 (IDH1)-mutant tumors form the majority of LGG, qualifying as a new diagnostic entity in the 2016 WHO classification of CNS tumors. Mutations in the tumor-defining enzyme IDH1 result in the increased production of R-2-hydroxyglutarate (R-2-HG) and constitute a distinct, metabolically skewed biological entity. These tumors are associated with less abundant and phenotypically altered immune cell infiltrates compared to IDH1 wild-type tumors. Despite recent advancements, the mechanisms shaping the immune microenvironment of IDH1-mutated tumors remain elusive. We could show that IDH1-mutated gliomas subdue their innate immune microenvironment by prompting a multifaceted reprogramming of myeloid cell metabolism. Integrated single-cell transcriptomic and proteomic analyses of human control and glioblastoma samples identified myeloid cell subsets with distinct fates in IDH1-mutated glioma that diverge from canonical trajectories of antigen-presenting cells. Paracrine tumor-derived R-2-HG, when transported into macrophages, induced an immunosuppressive state through dysregulated tryptophan metabolism and subsequent activation of the aryl hydrocarbon receptor (AHR), resulting in increased production of IL-10 and TGF-β, down-regulation of MHC-II expression, and consequent suppression of T cell activity. We demonstrate that effective immunotherapy in the context of mutant IDH1 requires normalization of this AHR-mediated phenotype. These findings argue for the development of new immunotherapy concepts that recognize the cell-specific immunomodulatory effects of IDH1-mutated tumors; and could prove vital defining the relevant entities targeted by small molecule AHR inhibitors currently undergoing preclinical development.

Keywords: Glioma, IDH, AHR, Tumor-associated myeloid cells.

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## A226 / Characterization of novel potent dual A2A/A2B adenosine receptor antagonists for cancer immunotherapy

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Adenosine (ADO) is one of the crucial metabolites responsible for immunosuppression in tumors. It is present in normal tissue in low concentrations, having various physiological functions, mainly prevention of too high immunostimulation which may cause tissue damage or even sepsis. However in the tumor microenvironment it's concentration significantly grows as the results of necrosis, hypoxia or inflammation as well as upregulation of CD39/CD73 proteins responsible for conversion of ATP to adenosine. Adenosine acts on various types of immune cells infiltrating the cancer tissue via either A2A receptor expressed for example on T lymphocytes, NK cells and M1 macrophages or via A2B receptor present on the surface of dendritic cells and M2 macrophages. Thus development of dual inhibitors is an attractive approach for cancer immunotherapy designed either as single agent or in combination with other immuno- or chemotherapeutics.

Here we present the characterization of equipotent dual A2A/A2B receptor antagonists that exert nanomolar potency in tumor-like adenosine-rich environment. Efficacy of compounds was confirmed in the in vitro assays based on cAMP modulation both in cell lines overexpressing A2AR or A2BR and in primary cells. Inhibition of adenosine dependent signaling pathway was confirmed in human whole blood assay in which phosphorylation of CREB (cAMP response element-binding protein) was the readout. Reversal of immunosuppression was confirmed in functional assays e.g. the cytokine release by activated CD4+ and CD8+ human T-lymphocytes, macrophages and dendritic cells. ComParison of new dual A2A/A2B inhibitors to the reference compounds currently tested in clinical trials showed superior efficacy of our compounds in functional in vitro models.

Keywords: Cancer immunotheraphy, Immune tolerance, Immunometabolism.

References:
Patent application - US2018265467 (A1)

## A227 / Investigate the role of T lymphocytes in the regulation of energy expenditure and obesity

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The increasing incidence of obesity and its co-morbid conditions poses a great challenge to global health. It is becoming clear that obesity is associated not only with type 2 diabetes mellitus and cardiovascular disease, but also with multiple types of cancer. Obesity and its associated inflammatory response has been closely linked to many aspects of cancer biology, including tumorigenesis, cancer immunotherapy and cancer cachexia. Obesity is caused by energy surplus when energy intake exceeds energy expenditure. As an important contributor of total energy expenditure is adaptive thermogenesis in brown adipose tissue, a promising and emerging avenue for obesity treatment is to increase energy expenditure by augmenting the activity of thermogenic adipocytes. Emerging evidence indicates that the immune system is instrumental in the control of host energy metabolism. However,

the underlying mechanism has not been fully understood; specifically, the role of T-cells in regulating host thermogenesis has not been well studied. Here we identified that gamma delta T-cells and their associated signaling play a critical role in mediating adaptive thermogenesis in response to cold exposure in brown adipose tissue and high-fat-diet-induced obesity. In pursuing these studies we hope to discover new ways (targeting immune cells) to support the obesity treatment and reduce obesity-associated systemic inflammation and complications, especially cancer.

Keywords: Immunometabolism, Obesity, Adaptive thermogenesis, T cell.

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## A228 / Immunometabolism modulation by PSGL-1 signaling in tumor-specific T cells

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P-selectin glycoprotein ligand-1 (PSGL-1) is an adhesion molecule expressed on the surface of naïve, effector, and memory CD4+ and CD8+ T cells. We identified PSGL-1 to be an immune checkpoint inhibitor as antibody-mediated ligation of PSGL-1 promotes T cell exhaustion and deletion of PSGL-1 prevents chronic viral infection and inhibits tumor. To investigate the role of PSGL-1 signaling in the development of T cell responses, we assessed the differentiation state, expansion capacity, and glycolytic profile of PSGL-1-deficient T cells. In a B16-OVA tumor model, we observed increased IL-13+ CD4+ T cells among the intratumoral T cell compartment in PSGL-1-/- mice. In vitro activation with a sub-optimal dose of CD3 of PSGL-1-/- OT-II CD4+ T cells demonstrated increased expansion and expression of CD25 compared to wild-type OT-II CD4+ T cells. Adoptively transferred in vitro-activated PSGL-1-/- CD4+ T cells demonstrated greater expansion in vivo upon adoptive transfer into RAG-/- host mice. Using the Seahorse glycolysis stress test, we identified that both CD4+ and CD8+ PSGL-1-/- T cells demonstrate increased glycolysis after 72 hours of in vitro activation compared to wild-type T cells. Further, in situ activation of PSGL-1-/- CD8+ T cells demonstrates that at both sub-optimal and optimal levels of CD3 stimulation, PSGL-1-/- CD8+ T cells have increased glycolysis and increased glycolytic capacity. Single-cell RNA-sequencing of tumor infiltrating PSGL1-/- CD8+ T cells shows the upregulation and modulation of several genes associated with T cell metabolism and enhanced intratumoral responses, suggesting an important role for PSGL-1 signaling in the development of effective anti-tumor T cell responses. Taken together, these data show that PSGL-1 signaling has an intrinsic and immediate role in the development of T cell responses and their metabolic profile.

Keywords: T cells, T cell exhaustion, Cancer, Glycolysis.

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## A229 / Metabolic intervention of tumor infiltrating lymphocytes with interleukine-10 leads to eradication of established solid tumors in mice

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Adoptive T-cell transfer (ACT) immunotherapy has produced stunning clinical results recently. Despite the great success in treating haematological malignancies, solid tumor remains a major challenge for ACT and cures remain rare. It has been reported that tumor-infiltrating lymphocytes (TILs) show a gradual loss of effector functions and proliferation capacity in the tumor microenvironment (TME), defined as T cell 'exhaustion'. From a metabolic point of view, glucose deprivation and other metabolic stress in the TME greatly alters T cell signaling and impair their antitumor immune response. Metabolic reprogramming of adoptively transferred T cells through ex vivo pretreatment has been shown to enhance ACT immunotherapy. However, a facile and safe in vivo metabolic intervention that can be combined with ACT to eradicate solid tumors and induce durable cures is still lacking. Interleukine-10 (IL-10), a member of the IL-10 family cytokines, which targets both innate and adaptive immune responses, is generally considered immunosuppressive as it reduces tissue damage caused by uncontrolled inflammatory responses. Recently, accumulating evidence has revealed the role of metabolic programing of IL-10 in macrophages. Here, we show that metabolic reprograming of tumor infiltration lymphocytes with IL-10/Fc markedly enhanced the efficacy of ACT against established solid tumors in both syngeneic and xenograft mouse models. Strikingly, up to 90% of mice bearing B16F10 mouse melanoma, a representative of an immunosuppressive, poorly immunogenic tumor, which received ACT and IL-10/Fc combination therapy, were cured and exhibited long-lived protective T-cell memory response against tumor re-challenging. We found that treatment with IL-10/Fc significantly expanded tumor infiltrating CD8+ T cells. In particular, the PD-1+TIM-3+CD8+ T cell subset was expanded to 12.9 and 5.9-fold higher number compared to mice without IL-10/Fc treatment in adoptively transferred and endogenous CD8+ T cell, respectively. The PD-1+TIM-3+CD8+ T cells were shown to vigorously produce granzyme B, IFN-$\gamma$, and TNF-$\alpha$ exhibiting enhanced effector functions for tumor cell killing. The mechanism studies revealed that IL-10/Fc modulated the metabolic fitness of tumor infiltrating PD-1+TIM-3+CD8+ T cells by increasing the oxidative phosphorylation (OXPHOS) and decreasing glycolysis. Together, we demonstrated a novel metabolic intervention strategy using IL-10/Fc for highly effective ACT cancer immunotherapy and provided evidence for the metabolic regulation mechanism of IL-10 in T cells.

Keywords: Metabolic intervention, tumor infiltrating lymphocytes, Interleukine-10, Adoptive T-cell transfer.

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## A230 / F-18-FDG-Positron Emission Tomography (PET)/ Computer Tomography (CT) imaging uncovers immune checkpoint inhibitor therapy responder-specific metabolic changes in the bone marrow

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Efficient immune checkpoint inhibitor therapy (CIT) in cancer patients is depending on a systemic immune response, which is not clearly elucidated yet. Nowadays CIT is employed in the clinical routine in a widespread manner e.g. in metastatic melanoma patients. Unfortunately, a large percentage of these patients does not respond to CIT or even progress rapidly. Unfortunately, we still lack diagnostic methods to predict a CIT-induced efficient cancer targeting immune response. Consequently, the majority of cancer patients underwent CIT without induction of an efficient anti-cancer immune response. The bone marrow (BM) is a major source of erythrocytes as well as immune cells homing to the tumor during CIT. Proliferation and differentiation of immune cells within the bone marrow is accompanied with an enhanced glucose metabolism. Thus, we focused in this study on the glucose metabolism of the bone marrow with non invasive in vivo F-18-FDG-PET/CT imaging to uncover a CIT-induced efficient anti-cancer immune response.

Using a register study at the University Hospital of Tübingen (064 / 2013 BO1) we analyzed F-18-FDG-PET/CT data retrospectively from 39 patients with metastatic melanoma and available F-18-FDG-PET/CT scans before and after the start of therapy with CTLA-4 and/or PD-1 mAbs. We excluded patients with uveal melanomas due to their different biological behavior as well as patients with diffuse bone metastases, bone marrow carcinosis and previous external beam radiation therapy on bone marrow regions. In total, 20 patients with F-18-FDG-PET scans within 50 days before and 125 days after the start of therapy were identified. For the analysis of the bone marrow we employed a semi-automatic segmentation tool, which is able to isolate the signal of the trabecular bone from that of the surrounding tissues, in particular, from the cortical bone. We conducted separate analyses for the entire skeletal system (without the skull and outer extremities), the axial skeleton (vertebrae and the sternum) and the appendicular bones (humerus- and upper half of the femur shafts).

We observed a significantly higher baseline F-18-FDG uptake within the bone marrow of metastatic melanoma patients responding to CIT when compared to non-responding patients (entire skeleton; ratio to the Background: 1.730.2 vs. 1.130.2, $p<0.05$). Interestingly, this effect was detected in the axial but not in the appendicular bones (2.230.2 vs. 1.530.1, $p<0.01$). After onset of CIT, the uptake in the entire skeleton increased by 19.036.8 % in responders ($p<0.01$) but slightly decreased in non-responders (-5.934.8 %). In the axial skeleton, the F-18-FDG uptake increased clearly by 16.735.0 % in the responders, whereas a marked decrease was measured in the non-responders (-9.334.4%, $p<0.01$). In the appendicular bones, we observed a trend towards a CIT-induced increase in F-18-FDG uptake in responders (62.3342.2 % as well as 7.4323.6%), while the uptake of the non-responders remained relatively stable. When excluding patients with bone metastasis, comparable results for the differentiation of responders and non-responders were identified.

Here, we present a first in vivo study investigating CIT-induced changes in the glucose metabolism of the bone marrow. Thus, the widely available in vivo 18F-FDG-PET/CT might represent a novel promising tool for non invasive in vivo detection of an efficient anti-cancer immune responses.

Keywords: immune checkpoint inhibitor therapy, imaging, PET/CT, bone marrow.

## A231 / Impaired mitochondrial respiration improves macrophages re-polarization

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Cervical and ovarian cancer are still major causes of death. In both, high infiltration of immune suppressive M2-like macrophages is associated with a poor prognosis. Therefore, M2 macrophages cells could be considered as target for therapy intervention in the context of immunotherapies. Since phenotype and activity of M2 macrophages is related to their metabolic profile, we are focused on the manipulation of the metabolic pathways of macrophages to unleash and/or enhance their anti-tumoral activity. Thus, we selected drugs (FDA-approved or soon-to-be) that, by targeting metabolic pathways, can improve M2-to-M1 macrophages re-programming or prevent polarization into M2 phenotype. We found that in mouse macrophages drug P (FAO inhibitor) and drug V (mitochondrial failure inductor) increased repolarization induced by IFN-γ, pro-inflammatory cytokine secretion and NO production, as well as impaired mitochondrial respiration and increased glycolysis, a M1 macrophages feature. Furthermore, drug-treated repolarized macrophages displayed an increased cytotoxic activity against tumor cells as compared with IFN-γ-repolarized macrophages, showing functional repolarization. On the other hand, drug C (glutaminolysis inhibitor), drug H (PPAR-inhibitor) and drug T (FAO inhibitor) and drug P prevented IL-4-mediated M2 polarization as well as decreased pro-tumoral cytokines secretion, suggesting that different metabolic pathways determine macrophages polarization or repolarization. Next, we tested the effect of drug P on human macrophages, since it was the only drug that increased M2-to-M1 repolarization and prevented M2 polarization. Drug P impaired mitochondrial respiration in a lesser extent than what we observed in mouse macrophages. However, it was capable of preventing IL-4 mediated macrophages polarization and boost IFN-γ-mediated re-polarization. Our findings support the idea metabolic modulation of macrophages to prevent their M2 polarization or improve re-programming into M1, and thus their activity against tumor cells. Overall, this new knowledge can help to develop new treatments aiming to create a pro-inflammatory tumor environment that improves immunotherapy.

Keywords: Macrophages, polarization, repolarization, metabolism.

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## A232 / Targeting metabolism to improve response to immune-checkpoint inhibition in melanoma

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In recent years, immune checkpoint inhibition has changed the treatment landscape in advanced melanoma. Despite the unprecedented increase in long-time survival of melanoma patients, at least 40-50% of patients do not benefit from treatment. Reprogrammed metabolism in melanoma leads to immunosuppression, which may be a key factor in compromising response to immunotherapy. This study aims to target metabolic reprogramming using dichloroacetate (DCA), which is a pyruvate dehydrogenase kinase (PDK) inhibitor. PDK inhibits pyruvate dehydrogenase (PDH), an enzyme that catalyzes the conversion of pyruvate into acetyl coenzyme A. Thus, DCA promotes entry of pyruvate into the citric acid cycle, thereby reducing glucose consumption and lactate secretion. We hypothesize that DCA treatment will render the melanoma cells less glycolytic, facilitating a stronger antitumor immune response. A clinical trial using DCA in metastatic melanoma patients is scheduled to start in the Department of Medical Oncology later this year to test this hypothesis in patients. In vitro, we treated both melanoma cells and immune cells with DCA to determine the effect of DCA on metabolism and viability of these cells. In melanoma cells, DCA decreased protein expression of the inactive phosphorylated form of PDH. Glucose consumption, lactate secretion and cell viability were reduced after using high doses of DCA. Inhibition of phosphorylated PDH was minimal in T cells or peripheral blood mononuclear cells (PBMCs). Production of interferon-γ, a key moderator of cell-mediated immunity, was increased after DCA treatment in both T cells and PBMCs. However, DCA treatment decreased proliferation in T cells. To summarize, DCA inhibits melanoma cells metabolically and increases cytotoxicity of immune cells, but it also affects T cell proliferation. Therefore, caution is advised while using this drug to potentiate the response to immunotherapy.

Keywords: melanoma, metabolism, dichloroacetate.

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## A233 / Preventing ATP- and AMP-degradation by suppressing CD39 and CD73 expression in T cells with LNAplus™ ASOs results in different effects as compared to small molecule-mediated A2A receptor blockade

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It is known that high adenosine levels in the tumor microenvironment can contribute to the suppression of antitumor immune responses and the so called adenosine axis has emerged as promising therapeutic target to enhance antitumor immune responses. The ectonucleotidase CD39 degrades extracellular immune-stimulating adenosine triphosphate (ATP) to adenosine monophosphate (AMP) which is then degraded to immunosuppressive adenosine by CD73. Adenosine then can bind to adenosine receptors such as A2A receptor (A2AR). So far, a direct comParison of ATP, AMP and adenosine on the one hand and blocking CD39, CD73 and the A2A receptor on the other hand has not been thoroughly performed in a cell culture system using human T cells.

We investigated effects of ATP, AMP and the non-degradable adenosine analogue 5'-N-Ethylcarboxamidadenosine (NECA) on activated T cells using flow cytometry and enzyme-linked immunosorbent assay (ELISA). CD39 and CD73 expression were suppressed using locked-nucleic acid (LNA)plus™ antisense oligonucleotides (ASOs) and the A2A receptor was blocked using small molecule inhibitors that are already used in the clinic.

Addition of ATP to activated T cells resulted in a significant reduction of T cell proliferation that could be reverted by ASO-mediated suppression of CD39 and / or CD73 expression. Furthermore, addition of AMP to activated T cells resulted in a significant reduction of T cell proliferation that could be reverted by ASO-mediated suppression of CD73 but not of CD39. Accordingly, inhibiting CD39 and / or CD73 expression prevented adenosine production upon addition of ATP. In strong contrast, we could not observe an anti-proliferative effect of NECA in our cell culture systems and A2AR small molecule inhibitors could not revert the suppression of T cell proliferation after addition of ATP or AMP to T cells.

Taken together, we herein show that other factors than adenosine potentially play an important role in the suppression of T cell activity. It has recently been shown by us (1, LNAplus™ ASO-mediated CD39 suppression) and others (2, pharmacological blockade of the A2A and A2B receptor) that targeting the adenosine axis shows therapeutic promise in vivo. Our recent data show, that the blockade of different components of the adenosine axis can have unique effects on T cells. Therefore, a combination approach where different molecules that are part of the adenosine axis are targeted should be considered for clinical applications in the future.

Keywords: CD39, CD73, Adenosine, Antisense oligonucleotides.

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## A234 / Inhibition of tumor glycolysis cooperates with CTLA-4 blockade in counteracting the immunosuppressive tumor microenvironment

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Immune checkpoint blockade has been demonstrated to significantly extend survival of cancer patients across multiple tumor types, formally positioning immunotherapy as a viable option for the treatment of cancer1. However, durable clinical responses occur only in a limited fraction of patients, highlighting the need to deepen our understanding of the mechanisms underlying response and resistance to checkpoint blockade to guide the rational design of more effective combination immunotherapies2. Cellular energy metabolism reprogramming is a critical hallmark of cancer3. Tumor cells typically adapt to meet their high energy demand by constantly consuming glucose and producing lactate through glycolysis (Warburg effect). High glucose consumption and lactate production by tumor cells restrict nutrient availability in the tumor microenvironment for effector T cells, which also rely on glycolysis to replicate and function4, making tumor metabolism an attractive target to exploit in combination with immunotherapy.

According to this hypothesis, we found that expression of glycolysis-related genes is inversely correlated with infiltration of most immune cell types in tumors from immunotherapy-naïve patients. However, after CTLA-4 blockade, immune-related and glycolysis-related genes are more often co-expressed in tumors, suggesting that anti-CTLA-4 may favor immune cell fitness in highly glycolytic tumor microenvironments. We thus investigated whether direct blockade of tumor glycolysis could potentiate the activity of anti-CTLA-4 against highly glycolytic and metastatic tumors, using the murine mammary carcinoma 4T1 as a model system in immune competent animals. We modeled selective blockade of glycolysis in tumors by knocking down the glycolytic enzyme lactate dehydrogenase A (LDH-A) in luciferase-expressing 4T1 cells (LDH-A-KD), and then tested the responsiveness of 4T1 LDH-A-KD tumors to CTLA-4 blockade. To more closely simulate the clinical management of breast cancer, we administered anti-CTLA-4 using a neoadjuvant schedule and surgical resection of orthotopically implanted LDH-A-KD or control 4T1 tumors. We then followed metastasis formation by bioluminescence imaging and overall survival. Neoadjuvant CTLA-4 blockade more efficiently extended survival of LDH-A-KD-tumor-bearing mice compared to controls, achieving 75% complete tumor remission that was associated with the development of long-lasting anti-tumor immunologic memory. Analysis of tumor immune infiltrates revealed increases in T cells, in particular CD4+ T cells, and decreases in CD11b+ myeloid cells in LDH-A-KD versus control tumors. However, anti-CTLA-4 did not substantially potentiate these effects in the setting of LDH-A-KD tumors. Intriguingly, we observed that anti-CTLA-4 increased the production of IFN-gamma and TNF-alpha by CD4+ T cells, in particular in regulatory T cells (Tregs), selectively in LDH-A-KD tumors. We thus asked whether higher glucose availability in the setting of glycolysis-defective tumors (LDH-A-KD) and improved CD86/CD80 co-stimulation via CTLA-4 blockade could render Tregs functionally unstable and potentially more like effector cells. We found that blocking CTLA-4 promotes glucose uptake by Tregs in LDH-A-KD tumors in vivo and that anti-CTLA-4 inhibits Treg-mediated suppression as a function of glucose concentration and CD86 expression in vitro, suggesting that CTLA-4 may contribute to alter Treg metabolism away from glycolysis, which would otherwise compromise the immune suppression function of Tregs.

Taken as a whole, these results underscore a new mechanism of action of CTLA-4 blockade that can be exploited by targeting tumor glucose metabolism. Since inhibitors of tumor glycolysis are currently being developed, testing them in combination with CTLA-4 blockade is a logical next step.

Keywords: Glycolysis, CTLA-4, Regulatory T cells.

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## A235 / Preliminary results from a prospective clinical trial of using C11-labeled alpha-methyl tryptophan (C11-AMT) PET Imaging as a predictor of response to pembrolizumab in stage III/IV melanoma (MM).

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Background: Molecular imaging of biochemical pathways critical for effector T cell response other than glucose metabolism could predict response to PD-1 inhibitors. We have previously shown that high protein expression of distinct enzymes within the tryptophan metabolic pathway in stage III/IV melanoma may correlate with reduced abundance of tumor-infiltrating lymphocytes(1). We therefore investigated the significance of C11-AMT, a PET tracer that images tryptophan metabolism, prior to treatment as a predictor of response to pembrolizumab in patients (pts) with PD-1 inhibitor-naïve MM.

Patients/Methods: In this prospective biomarker-driven trial (2) pts must have had measurable disease by RECIST v1.1 criteria and must have undergone FDG-PET & C11-AMT PET imaging as well as a mandatory tumor biopsy prior to initiation of pembrolizumab. RESULTS: 19 pts (15 males; 14 stage IV; median age 61, range 30-90) had baseline FDG-PET and C11-AMT PET (dynamic imaging at 30-40min) studies performed. 12 pts were non-progressors (3 with complete response, 6 with partial response, and 3 with stable disease). The median follow-up of the 19 patients was 13 months (2.1-22.7+ months), 4 pts were dead from MM, 8 were alive with no evidence of MM and 7 pts are alive with MM. 43 individual tumor lesions were assessed by FDG-PET, C11-AMT, and CT scan with IV contrast. Of the pts with one or more tumor lesions certain C11-AMT PET parameters (SUVmax ≤ 7, and skewness ≤ +0.2) of the tumor lesion with the highest C11-AMT SUVmax were associated with non-progression by RECIST (SUVmax ≤ 7/skewness ≤+0.2 in progressors vs. non-progressors; Fisher's exact one-tail test p=0.04). There was a moderate but significant correlation between FDG-PET SUVmax and C11-AMT PET SUVmax among the 43 measured tumor lesions (Spearman rho=0.43, p=0.003).

Conclusion: Baseline C11-AMT PET imaging using simple measures of the area with the highest metabolic activity within the tumor (SUVmax) and histogram-derived texture parameters of tumor heterogeneity (skewness) may predict clinical benefit in pts with PD-1 inhibitor-naïve MM. Variability in C11-AMT's SUVmax cannot be completely explained by FDG-PET's SUVmax, suggesting that these two imaging modalities may provide complementary information about the metabolic dysregulation within the tumor that may be associated with immunotherapy response. Texture analysis using LifeX v4.0 and immunohistochemical analysis of research tumor biopsies for components of the tryptophan (TPH1, TPH2, IDO1, TDO2, LAT1) and glucose metabolism (GLUT1, HK1, HK3) will be presented at the meeting. Accrual is ongoing.

Keywords: C11-labeled alpha-methyl tryptophan, PET, pembrolizumab, metastatic melanoma.

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# Combination therapies with immune checkpoint blockers

## A236 / Development of a novel, highly effective tripartite immunotherapy using autologous humanized PDX models and genetically engineered mouse models of cancer

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Less than 10% of all cancer patients respond to immune-checkpoint-inhibitors and less than 30% to any form of immunotherapy. Therefore, novel approaches are needed and ought to be developed rationally using appropriate model systems. Genetically engineered murine cancer models can be used to develop immunotherapeutic interventions for cancer with the tumor being in its natural environment and subject to immunoediting. However, profound differences between human and murine immunity limit translation. In contrast, humanized mouse models of cancer enable the study of interventions affecting the human cancer-immune-interaction, but only in a xenogeneic host.

Here, we develop a novel combination immunotherapy of intratumorally delivered TLR agonists, aPD1-checkpoint-blockade and a multicellular therapy of innate and adaptive effectors utilizing genetically engineered mouse models of cancer and, importantly, autologous humanized PDX transplant mouse models of lung cancer.

Using TCGA-data, we found that a combined infiltration of activated NK-cells, γδT-cells and non-regulatory αβT-cells confers a uniform survival benefit across cancers, far exceeding the prognostic value of T-cell infiltration alone.

Thus, we adapted protocols to selectively expand NK-like-, γδT- and tumor-specific αβT-cells in vitro from human PBMCs or mouse splenocytes. Tumor-specific T-cells were induced in vitro in a coculture system with the respective tumor target. Experiments confirmed cytotoxicity, specificity, functionality and adequate expansion of subsets. We show that NK-like-, γδT- and tumor-specific αβT-cells act synergistically in combination to kill lung-, melanoma-, breast-, and lymphoid cancer cells and exhibit increased cytotoxic activity when exposed to aPD1-antibodies in vitro. Next, we performed in vivo experiments in humanized NSG-mice carrying lung- (H441, H1975), breast- (JimT1), and lymphoid (KMH2) cancers, in a syngeneic melanoma model (B16F10) and in a difficult to treat K-ras / p53 driven (KP) genetically-engineered mouse model of lung cancer as well as autologous humanized PDX lung cancer models. We sequentially combined intratumoral TLR agonist therapy targeting TLR-3, TLR-7 and TLR-9, a combined adoptive cellular therapy of NK-like-, γδT- and tumor-specific αβT-cells and aPD1 immune checkpoint blockade. Strikingly, we were able to show that all 3 elements are needed in order to effectively eradicate established tumors across models.

Aiming to elucidate mechanisms, we first enumerated and characterized immune cell subsets in tumors and spleens. Combination treatment leads to notable increases in tumor-infiltrating γδT-cells, CD4+ and CD8+ T-cells as well as NK-cells across models. Furthermore, we used multiplex cytokine arrays to evaluate both changes in cytokines associated with tumor regression and those associated with certain treatment combinations. Our most prominent finding was a pattern of increased cytokine levels of MIP-1a, IL-5, IL-4, IL-9 and IL-15 associated with both reduced tumor growth and combination immunotherapy. Cell subset depletions studies and functional studies showed multiple anti-tumor mechanisms at work, collaborating to eradicate established tumors. We could show induction of tumor specific, polyfunctional T-cells, a tumor-specific antibody response and dependence of treatment efficacy on presence of both innate and adaptive effectors.

In conclusion, we show that a tripartite immunotherapy of intratumorally delivered TLR agonists, aPD1-checkpoint-blockade and a multi-cellular therapy of innate and adaptive effectors is highly effective across cancer entities and model systems, including PDX models and genetically engineered mouse models of cancer. Importantly, we were able to develop a novel, highly effective, tripartite immunotherapy regimen with broad efficacy that is completely agnostic of the precise target antigen and thus translationally highly relevant.

Keywords: combination immunotherapy, cellular therapy, PDX models, local immunotherapy.

# POSTER SESSION B

## Cancer prevention and life style factors in oncoimmunology

### B001 / Investigating the influence of obesity on cancer immunotherapy

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Obesity is one of the main lifestyle-related factors causing cancer(1). In addition to contributing to cancer development, obesity promotes cancer recurrence and progression, while also reducing the efficacy of therapeutic interventions. In particular, cancer immunotherapies have been shown to be both less effective in obese animals and at the same time associated with greater toxicities(2, 3). In this project, we aimed to investigate if novel cancer vaccines, containing tumor antigens delivered on virus-like particles (VLP), could improve survival of obese and lean mice with breast or colorectal cancer.

We utilized two VLP constructs, both designed to recombinantly express the murine survivin peptide, with one containing an additional mucin1 peptide conjugation. For the breast cancer model, we utilized an orthotopic model, with C57.mg breast cancer cells injected into the mammary fat pad of C57Bl/6 and obese Pound mice. VLPs expressing survivin only were investigated in a colorectal cancer model where tumor formation was induced by subcutaneous injection of MC-38 cells. Once tumors were palpable, mice were vaccinated with VLPs expressing survivin and mucin1 (breast cancer model) or survivin alone (colorectal cancer model), both given in combination with the vaccine adjuvant CpG. Additionally, we investigated whether obesity-associated immune suppression could be alleviated, by treating mice with a combination of VLPs and anti-PD-L1 immune checkpoint blockade. Repeat administration of anti-PD-L1 was conducted on day three and six following initial treatment. Tumor growth, body weight and overall well-being of mice were monitored to assess the efficacy and tolerability of the treatments. To investigate potential underlying mechanism for differences in the immune responses of obese and lean mice, the composition of bone marrow cell populations in the different mouse strains was assessed by flow cytometry. Furthermore, the ability of bone marrow cells, differentiated into bone marrow-derived dendritic cells (BMDCs), to respond to stimulation with the vaccine adjuvant CpG was investigated.

We observed that obese mice had higher levels and total numbers of CD11b+ myeloid cells and immature myeloid cells (CD11b+ Ly6C+ Ly6G+ cells and CD11b+ Ly6C+ Ly6G- cells) in the bone marrow. When bone marrow cells were differentiated into BMDCs and stimulated with CpG we found a decrease in expression of MHC-II, CD40 and CD86 in BMDCs from obese mice compared to lean mice, indicating a decreased responsiveness to adjuvant stimulation. Obese and lean mice challenged with either colorectal or breast cancer cells had similar tumour growth kinetics when left untreated or treated with the VLP constructs. However, differences were observed in groups of mice treated with the combination of a VLP and PD-L1 immune checkpoint blockade, indicating that VLPs given in combination with checkpoint blockade may be less effective at delaying tumour growth in obese mice as compared with wild type littermates. Taken together, our data indicate that obese mice were less responsive to combination therapy with VLPs and immune checkpoint blockade, with further studies needed to delineate the underlying mechanisms.

Keywords: cancer immunotherapy, obesity, breast cancer, colorectal cancer.

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### B002 / Developmental programming of long-term immunity of CD8 T cells by perinatal glucocorticoid

Jun Young Hong (Yale University), Ruslan Medzhitov (Yale University).

Stress has been associated with various types of diseases including cancer. It was suggested that compromised anti-tumor immunity is often responsible for tumor progression during stress, which is caused by immunosuppressive glucocorticoid (GC), a stress hormone, and sympathetic nervous system activation. Perinatal period is critical for immunity as the first major contact with the environment is made and this interaction can shape the immune system development. Epidemiologic studies found that early-life exposure to specific environment may have life-long impact on immunity, affecting the development of immune-related diseases such as inflammatory diseases, metabolic diseases, allergic diseases as well as cancer. Nevertheless, it is still largely unknown whether developmental programming of immunity exists due to the lack of mechanistic understanding on this subject.

Since most of the early environmental factors that are reported to cause later-life development of diseases are associated with stress, we adopted a model to directly test the effect of stress hormone. We introduced the in vivo mouse model of perinatal glucocorticoid receptor (GR) activation via dexamethasone (DEX) treatment in drinking water perinatally (embryonic day 7.5-postnatal day 1). Then, we analyzed the T cell immunity when the offspring became mature. We found that perinatal GC exposure elicited diminished CD8 T cell response in adulthood. As a result, mice with perinatal GC treatment showed insufficient anti-tumor and anti-bacterial CD8 T cell responses, being susceptible to immunogenic tumor development and Listeria infection. We found significantly reduced systemic levels of corticosterone (CORT) in the adult mice with perinatal GC exposure. Deficiency of GR signaling in CD8 T cells with adrenalectomy or with genetic deletion of GR in T cells was sufficient to reduce CD8 T cell response. Inhibition of GR signaling in CD8 T cells acted primarily on CD25 signaling and mTOR pathway, resulting in decreased effector function and survival. Finally, we found the negative feedback threshold of HPA axis is altered by having enhanced mineralocorticoid receptor (MR; type I corticosteroid receptor) expression in the hippocampus and paraventricular nucleus of hypothalamus (PVH) with perinatal GC exposure. Our study shows that perinatal stress may have long-term consequences on CD8 T cell immunity by altering HPA axis threshold, thereby increasing predisposition to cancer and bacterial infection.

These results showed that perinatal GC exposure persistently programed the threshold for hypothalamus-pituitary-adrenal axis for regulating endogenous GC level, and that reduced systemic GC level elicited repressed CD8 T cell activation and survival, leading to tumor susceptibility.

Keywords: Glucocorticoid, Early-life stress, CD8 T cells.

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Okin, D., and Medzhitov, R. (2012). Evolution of inflammatory diseases. Curr Biol 22, R733-740.

### B003 / Low-dose carbon ion radiation boosts anti-tumor immune response by inhibiting myeloid-derived suppressor cell (MDSC) proliferation through JAK2/STST3 signaling pathway in melanoma bearing mice

Heng Zhou (Institute of Modern Physics, Chinese Academy of Sciences).

Compared with conventional radiotherapy, heavy ion beams have distinct advantages in cancer therapy due to their high-energy sharp Bragg peak and potent killing effect on tumor cells. Here, we report that in situ a single fraction of low-dose carbon ion radiation reduces the number of MDSC in the bone marrow, peripheral blood and spleen of melanoma bearing mice through a JAK2/STAT3-dependent mechanism. In contrary the percentage

of CD4+ CD8+ T cells, macrophages and natural killer cells increases which results in reduced tumor growth and prolonged overall survival of mice. In summary we show that single fraction low-dose carbon ion irradiation can boost anti-tumor immune responses via the inhibition of MDSC.

Keywords: Carbon ion radiation, MDSC, JAK2/STAT3 signaling pathway.

## B004 / Infiltration of metastatic lymph nodes with PD-1+ T cells is associated with improved disease-free and overall survival in resected N+ NSCLC

shuo Wang (Capital Medical University Shijitan Hospital).

Purpose: Tumor metastases to regional lymph nodes are associated with worse outcome for patients with resected non-small cell lung cancer (NSCLC), but there is a wide variation in survival. We hypothesize that infiltration of tumor-involved lymph nodes with activated effector T cells is associated with improved prognosis.

Methods: A total of 28 lymph nodes (18 N+ and 10 N-) collected from 7 Stage T2N1M0 patients and 4 lymph nodes collected from 3 Stage T2N0M0 patients who underwent lymphadenectomy during surgical management of their NSCLC were analyzed for effector T cells expressing the activation markers PD-1 and TIM-3 using the Opal-multiple immunofluorescence assay.

Results: The frequency of CD3+CD8+(P=0.02), CD3+CD8+-TIM-3+ (P=0.0045), CD3+CD8+TIM-3+Ki-67+(P=0.0017) T cells were greater in lymph nodes of N0 patients compared with N1 patients; however the frequency of CD3+CD8+PD-1+(P=0.039), CD3+CD8+PD-1+Ki-67+ (P=0.025) and CD3+CD8+TIM-3+Ki-67+ (P=0.025) T cells was greater in the tumor involved (N+) nodes of N1 patients compared with the tumor-uninvolved (N-) nodes. The frequency of intranodal CD3+CD8+, CD3+CD8+PD-1+ and CD3+CD8+PD-1+Ki-67+ T cells in N+ nodes was associated with prolonged progression-free (PFS) and overall survival (OS).

Conclusion: CD3+CD8+TIM-3+ T cells may control tumor spread to regional lymph nodes but once tumor has spread to lymph nodes, CD3+/CD8+/PD-1+/Ki67+ T cells localized within N+ nodes may prevent some tumor cells from invading further resulting in prolonged survival.

Keywords: NSCLC, Metastatic lymph node, T cell subsets, PD-1.

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## B005 / The prevalence and associated factors of cigarette smoking and its association with opium use among outpatients in Afghanistan: a cross-sectional study in Andkhoy city

Mohammad Hassan Hamrah (Andkhoy).

The objectives of this study were to estimate the prevalence and associated factors of cigarette smoking and the association between cigarette smoking and opium use among patients visiting an outpatient clinic in Afghanistan.

Methods: A cross-sectional study was conducted on consecutive patients aged 18 years and older from January 2018 to April 2018. Data on patients' sociodemographic characteristics and clinical variables were collected using an interview-based survey. Results: Six hundred and twenty-two patients (391males vs. 231 females) were interviewed for this study. The overall prevalence of current smoking was 50.2% (95% confidence interval [CI]: 46.2–54.2). Males were (odds ratio [OR] = 9.5; 95% CI: 5.3–17.1) more likely to smoke cigarettes than females. The odds of current cigarette smoking increased with having a family member smoker or a friend smoker (OR =3.3; 95% CI: 2.0–5.3). Cigarette smoking was significantly associated with the level of education (illiterate OR = 8.9; 95% CI: 4.0–19.8), primary/private education (OR = 7.8; 95% CI: 3.9–15.6), and secondary education (OR = 4.4; 95% CI: 2.3–8.4), with high school or higher education as the reference group. Rural residents were 3.7 times (95% CI: 2.3–6.2) more likely to smoke cigarette than urban residents. Opium users were 23.0 times (95% CI: 12.5–42.3) more likely to smoke cigarettes than non-opium users.

Conclusion: The prevalence of cigarette smoking among patients visiting an outpatient clinic in Afghanistan was high, and there was an association between cigarette smoking and male gender, a family history of smoking or a friend history of smoking, level of education, rural residency, and opium consumption.

Keywords: Cancer, Andkhoy, associated factors, smoking.

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## B006 / Controlled dietary interventions in patients with melanoma

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There is increasing evidence from our group and others that host factors can influence melanoma biology, the anti-tumor immune response, and patient outcomes. In preclinical models, we have shown that dietary modulation of systemic insulin and IGF-1 can influence IGF-1R/PI3K/AKT pathway activation and melanoma tumor growth. In observational cohorts, melanoma patients who reported higher baseline consumption of fiber-rich plant foods were five-times more likely to respond to anti-PD1 immunotherapy, and have higher abundance of gut bacteria we have previously found to correlate with anti-PD1 response. While dietary interventions in other populations have been shown to successfully modulate these two melanoma-relevant targets (insulin/IGF-1 and the gut microbiome), to our knowledge, dietary studies have never been conducted in patients with melanoma. We hypothesize that controlled dietary interventions will be feasible in a melanoma population and will impact key biological processes relevant to melanoma therapeutic response. The trial is a single-institution fully-controlled feasibility feeding study of two diets in parallel: (1) a high-fiber diet targeting the gut microbiome and (2) a ketogenic diet aimed at lowering systemic insulin/IGF-1. Up to 20 patients with a history of melanoma within the last five years, without evidence of disease, and off systemic therapy at least one year will be assigned in blocks of two patients to each diet. All calorie-containing food and beverages will be provided by the MD Anderson Bionutrition Research Core for the duration of the six week study. Both diets will be isocaloric to patient's usual calorie intake/needs. Fiber in the high-fiber diet will be whole-foods derived from diverse whole grains, fruits, and vegetables will otherwise comply with the standard American Institute for Cancer Research diet recommendations. Fiber content begins at 30 g and will be titrated up to 50 g daily as determined by tolerability. The ketogenic diet is a high-fat, moderate-protein, low-carbohydrate macronutrient profile. Fat sources in the ketogenic diet will be high-quality, beginning at 65% (3:1 fat: carb + protein) and will be titrated up to 82% (4:1) as determined by tolerability. Blood and fecal specimens will be collected q2 weeks. The primary endpoint is feasibility, defined by ≥25% of eligible patients enrolling. Secondary clinical endpoints include adherence (≥70% of calories consumed from provided diets assessed by dietary records), safety, and tolerability. Correlative endpoints include changes in gut microbiome (composition, diversity, and metabolic function), systemic insulin and IGF-1 levels, and systemic immune parameters (PBMC flow and serum cytokines). To date, two patients have been accrued to the study. results from this trial will be used to inform the design of dietary intervention studies in patients receiving systemic therapy. The high-fiber diet will be tested in patients with metastatic melanoma receiving anti-PD1 immunotherapy with the goal of modulating the microbiome and enhancing systemic and anti-tumor immunity. Based on preclinical data demonstrating synergy between the ketogenic diet and PI3K pathway inhibitors through prevention of hyperinsulinemia/hyperglycemia, the ketogenic arm will be expanded into patients receiving targeted therapy. Ultimately, multi-institutional Phase III trials powered to test the effects of dietary modification on cancer outcomes will be conducted in a broader population via nutritional counseling/behavioral intervention.

Keywords: Melanoma, diet, microbiome, immunotherapy.

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# Combination therapies with immune checkpoint blockers

## B007 / Development of small molecule PD-L1 inhibitors

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Inhibition of PD-1/PD-L1 interaction by antibody has obtained great success in cancer immunotherapy. Ten PD-1/PD-L1 mAbs with more than 10 different indications were approved by FDA or NMPA in U.S. or China. While the progress of small molecule PD-L1 inhibitor is slow. Most researchers considered that small molecule inhibitor could not achieve comparable binding affinity to mAbs, so it is less competent with mAbs. Until recently, researchers from Incyte Corporation reported that their small molecule PD-L1 inhibitors INCB86550, which entered the clinical trial phase1 in the end of 2018, could induce internalization of PD-L1 on cell membrane, which is different from antibodies. As small molecule PD-L1 inhibitor has such distinct mechanism, it is possible to realize the differentia competition with mAbs. Hence, development small molecule PD-L1 inhibitors can be an effective supplement to immune checkpoint blockade strategy. Here, we describe our recent work in discovery of small molecule PD-L1 inhibitors. In our study, we identified a series of biphenyl derivatives, which have moderate to potent PD-1/PD-L1 inhibition activity with IC50 values of nano-molar to submicro-molar. In mixed lymphocyte reaction assay, our representative compound ZJU-003 could significantly enhance T-cell function through blockade of PD-L1. In T cell killing assay, ZJU-003 could increase the sensitivity of tumor cells (MDA-MB-231) to T cell killing. The PK characters of ZJU-003 were also evaluated, ZJU-003 exhibited moderate oral bioavailability. The anti-tumor activity of ZJU-003 in PD-1/PD-L1 humanized mice is still undergoing.

Keywords: PD-L1, SMALL MOLECULE INHIBITOR.

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## B008 / Crizotinib-induced immunogenic cell death in non-small cell lung cancer

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Immunogenic cell death (ICD) converts dying cancer cells into a therapeutic vaccine and stimulates antitumor immune responses. Here we unravel the results of an unbiased screen identifying high-dose (10 QM) crizotinib as an ICD-inducing tyrosine kinase inhibitor that has exceptional antineoplastic activity when combined with non-ICD inducing chemotherapeutics like cisplatin. The combination of cisplatin and high-dose crizotinib induces ICD in non-small cell lung carcinoma (NSCLC) cells and effectively controls the growth of distinct (transplantable, carcinogen- or oncogene induced) orthotopic NSCLC models. These anticancer effects are linked to increased T lymphocyte infiltration and are abolished by T cell depletion or interferon-γ neutralization. Crizotinib plus cisplatin leads to an increase in the expression of PD-1 and PD-L1 in tumors, coupled to a strong sensitization of NSCLC to immunotherapy with PD-1 antibodies. Hence, a sequential combination treatment consisting in conventional chemotherapy together with crizotinib, followed by immune checkpoint blockade may be active against NSCLC.

Keywords: immune checkpoint blockers, immunotherapy, tyrosine kinase inhibitor, off-target effects.

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## B009 / FS118, a LAG-3/PD-L1 bispecific antibody, capable of driving potent anti-tumour immune responses and overcome PD-(L)1-mediated compensatory upregulation of LAG-3 induced by single-agent checkpoint blockade

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FS118, currently being evaluated in a Phase 1 clinical trial in patients with advanced malignancies (NCT03440437), is an IgG1 mAb2 tetravalent bispecific antibody targeting LAG-3 (Lymphocyte-Activation Gene 3) and PD-L1 (Programmed Death-Ligand 1), two immune checkpoint molecules that promote tumour escape from immune surveillance.

Despite advances with therapies targeting the PD-(L)1 pathway, many patients are refractory or relapse following treatment. Resistance to anti-PD-1 treatment is associated with upregulation of other checkpoint inhibitor receptors such as LAG-3. The expression of LAG-3 on exhausted T cells and T regulatory cells (Tregs) in the tumour may be responsible for this resistance and provides a rationale for co-treatment with antibodies targeting LAG-3 and PD-L1. Dual targeting these immune checkpoint blockade (ICB) using bispecific monoclonal antibodies could potentially overcome this resistance, further increase the therapeutic benefit of ICB therapy and prevent relapse or resistance after immunotherapy. Bispecific antibody-based immunotherapeutic strategies have the potential to deliver superior activity to combination therapy through novel mode of action such as tumour redirected T cell activation, simultaneous checkpoint regulators targeting, increased modulation of receptor signalling and target engagement through avidity binding effects.

FS118 was engineered by introducing a distinct bivalent LAG-3 binding capability into the CH3 region of the Fc portion of a human IgG1 molecule and assembled into a bispecific format with anti-PD-L1 Fabs. Additional mutations introduced into the CH2 domain of the Fc region suppress Fc-mediated effector function. FS118 was evaluated in vitro for antigen binding and in a super-antigen stimulated peripheral blood mononuclear cells (PBMC) assay. Anti-tumour activity of a murine-specific molecule, mLAG-3/PD-L1 mAb2 (FS118 surrogate), was assessed in vivo in the MC38 mouse tumour model and associated immunophenotypic changes were evaluated using flow cytometry.

FS118 has two binding sites for both LAG-3 and PD-L1. It is capable of binding to both targets simultaneously and can de-repress the inhibitory function of human PD-L1 and human LAG-3 in a human PBMC assay, as measured by cytokine, with at least equivalent activity to a combination of anti-LAG-3 and anti-PD-L1.

In a syngeneic model, a FS118 surrogate was used to determine the effect of dual checkpoint blockade on tumour growth and the modulation of LAG-3 and PD-L1 expression on tumour infiltrating lymphocyte (TIL) following treatment with the mAb2. Dual blockade of LAG-3 and PD-L1 with the surrogate mAb2 in the MC38 tumour model resulted in an increased anti-tumour activity comparable to a combination of the single agents. Moreover, the mAb2 and single agent combination resulted in distinct modulations of LAG-3 and PD-L1 cell surface expression on TILs. While both the mAb2 and combination therapy significantly reduced the number of free PD-L1 binding sites on CD4+ and CD8+ T cells on TILs, total LAG-3 cell surface expression increased following treatment with the combination, whereas it was reduced with mAb2 treatment. In addition, analysis of serum samples collected at various timepoints confirmed evidence of drug target engagement with increasing levels of both soluble LAG-3 (sLAG-3) and soluble PD-L1 (sPD-L1) in the mAb2 treated animals.

Dual blockade of LAG-3 and PD-L1 with FS118 resulted in T cell activation at least comparable to a combination of antibodies targeting LAG-3 and PD-L1 in primary T cell assays and murine tumour models. These data support the rationale for clinical development of FS118 for the treatment of cancer patient with adaptive or acquired resistance to PD-(L)1 therapy.

Keywords: LAG-3/PD-L1 bispecific mAb, Immunotherapy, PD-1 resistance.

## B010 / An oncolytic adenovirus mediated by PD-1 antibody for cancer therapy

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Oncolytic viruses (OV) are native or recombinant viruses which preferentially target and destroy cancerous tissues without causing damage to normal cells. In addition to T-VEC, the first OV-based drug approved by the Food and Drug Administration, adenovirus is widely used in preclinical and clinical trials and demonstrates therapeutically anticancer promise. On account of the programmed cell death protein 1 (PD-1) limits effector T cells function upon interaction with the ligand, PD-L1 or PD-L2, and promotes tumor own growth, therapy with anti-PD-1 has been indicted to strengthen the immune response in multiple malignancies, while a majority of patients lack response to single immune check point inhibitor due to the deficient tumor immunogenicity, inadequate infiltrated T cells and other unknown factors. Hence, we plan to exploit a novel oncolytic adenovirus AdC68-sp/E1A-nivolumab, which is armed with full-length human PD-1 monoclonal antibody (nivolumab). On one hand, the oncolytic virus could elicit and recruit abundant T cells to disrupt the suppressive tumor microenvironment and activate effector T cell to recognize and disintegrate tumor cells. On the other hand, wherein the PD-1 monoclonal antibody can effectively produce and play a critical role in tumor microenvironment. The study in vitro indicated that the insertion of PD-1 didn't change the oncolytic properties of OV and the PD-1 monoclonal antibody was expressed with biological function in a panel of tumor cells like Huh-7, Siha, NCI-H508, A549. Meanwhile, the viability of tumor cells treated with AdC68-sp/E1A-nivolumab descended poignantly in a mechanism of virus-induced apoptosis through activating the p53 signaling pathway and cell cycle retardation of cancer cells. Further study in immunocompetent and immunodeficient mouse models is underway to evaluate the synergistic anti-tumor efficacy and mechanism in vivo.

Keywords: oncolytic adenovirus, human PD-1 monoclonal antibody, cancer therapy.

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## B011 / Intratumoral, but not intravenous, CD40L/4-1BBL virotherapy (LOAd703) induces systemic anti-tumor immune responses and enhances the effect of PD-L1 checkpoint blockade

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The purpose of this study was to evaluate the capacity of stimulating a systemic anti-tumor immune response using local or systemic injections of an oncolytic adenovirus encoding TMZ-CD40L and 4-1BBL (LOAd703). Moreover, the possibility to further enhance responses by combining LOAd703 with PD-L1 checkpoint blockade was investigated. Immune checkpoint inhibitors have changed the outlook of numerous cancer patients. However, most patients are resistant to treatment, which is associated with a non-inflamed tumor phenotype. A promising clinical approach is to convert such tumors into inflamed tumors by using oncolytic viruses combined with checkpoint blockade. Herein we evaluated LOAd703, with and without PD-L1 blockade, in preclinical melanoma models. A murine version of LOAd703 (mLOAd703) expressing murine TMZ-CD40L and 4-1BBL was evaluated in the B16-hCD46/C57BL6 model while LOAd703 with human transgenes was used in human DC/526-mel co-culture experiments. Immune activation was determined by flow cytometry and multiplex assays from Meso Scale Discovery. Note that adenoviruses do not replicate in murine cells, hence, the treatment effect of mLOAd703 in animal models is only due to expression of the immunostimulatory transgenes. A twin-tumor model was used to evaluate the effect of mLOAd703 (1x10e9 ffu) by repeated intravenous (i.v.) or intratumoral (i.t.) injections (one lesion). I.v. treatment did not control tumor growth. In contrast, i.t. treatment could control growth of the injected tumor as well as hamper growth of the non-injected tumor demonstrating that it is more effective to induce immunity by creating a highly active site (high virus load) than to reach all lesions with a small number of virus particles. Dendritic cells (DCs) play a central role in mediating systemic immune responses and require adequate activation to function properly in the tumor microenvironment. In a human melanoma/DC co-culture model, LOAd703 activated DCs to express a wide range of co-stimulatory molecules (e.g. CD80, CD86) as well as the lymph node homing receptor CCR7. Extensive DC activation concurrently upregulated PD-L1 expression, which underlines the relevance of checkpoint blockade combination. To test this combination, mice with twin-tumors were treated with i.t. mLOAd703 (1x10e9 ffu), anti- PD-L1 (intraperitoneal 5 mg/kg/injection), a combination of both, or treated with an isotype control antibody (i.e negative control). Anti-PD-L1 monotherapy had only a limited effect on tumor growth, whereas the combination with mLOAd703 could control the growth of the injected tumor and further delay the growth of the non-injected tumor compared to mLOAd703 alone. In agreement, flow cytometry analysis of the tumor biopsies showed a significant increase of CD8+ T cells in both tumors (Non-injected vs injected: mean % CD8+ of CD45+ cells; Isotype control: 4 vs 3%, anti-PD-L1: 7 vs 9%, mLOAd703: 8 vs 14%, combination: 19 vs 20%). Likewise, serum levels of T effector cytokines such as IFNg and TNFa were highest in animals treated with the combination.

In conclusion, local but not systemic CD40/4-1BB-stimulating virotherapy induced a systemic anti-tumor immune response in the non-immunogenic B16-hCD46 melanoma model. The effect of local therapy was further increased by anti-PD-L1 which did not show a significant effect as monotherapy, meaning that LOAd703 could sensitize otherwise resistant mice to PD-L1 checkpoint blockade. LOAd703 is currently under clinical investigation for the treatment of solid tumors (NCT03225989) and pancreatic cancer (NCT02705196). The latter trial will be expanded to include combination with anti-PD-L1. In addition, a trial investigating the combination in checkpoint-resistant melanoma patients will be initiated shortly.

Keywords: Oncolytic virus, Checkpoint blockade, CD40L, 4-1BBL.

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## B012 / Axl inhibition improves immunotherapy by targeting local and systemic tumor-myeloid crosstalk

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Tumor mobilization of suppressive myeloid cells is a primary obstacle to immune checkpoint inhibitor (ICI) efficacy. Here we show that combining ICI with the small molecule AXL kinase inhibitor bemcentinib improved durable response by targeting tumor-immune crosstalk in a refractory suppressive myeloid-dominated murine adenocarcinoma model. ICI/bemcentinib therapy blocked ICI-induced tumor AXL and Colony Stimulating Factor 1 (CSF1) expression and acquired EMT plasticity markers. High dimensional mass cytometry immune profiling demonstrated that AXL-expressing peripheral and tumor microenvironment resident suppressive myeloid cells were targeted by bemcentinib treatment. We reveal that AXL defines novel splenic and tumor dendritic cell subpopulations characterized by immune suppressive marker expression. AXL targeting by bemcentinib blocked tolerogenic dendritic cells and enhanced adaptive anti-tumor immunity. Our findings indicate that AXL signaling integrates cancer cell plasticity with immune suppressive myeloid and regulatory dendritic cell mobilization and that tumor immunity can be enhanced by combined ICI and AXL targeting.

Keywords: AXL, Bemcentinib, High dimensional mass cytometry immune profiling (CyTOF), Immune suppression.

## B013 / Neoadjuvant immunotherapy followed by cancer surgery re-sets the tumor-specific T cell response to provide durable protection against residual metastatic disease

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Neoadjuvant immunotherapy followed by cancer surgery has shown improved efficacy over adjuvant immunotherapy, but the mechanism of action underlying this effect is not fully understood. Using a mouse model of spontaneous metastatic cancer we have shown that neoadjuvant immunotherapy can "reset" the anti-tumor immune response more akin to that which occurs following an acute viral infection; with discreet re-expansion, contraction, and memory differentiation phases. Compared with adjuvant-treatment, neoadjuvant-treatment improved the quality of tumor-specific CD8+ T cells as characterized by their increased effector cytokine production and in vivo cytotoxicity. These effects were dependent on the timely resection of the primary tumor and independent of de-novo tumor-specific CD8+ T cell priming following treatment. This understanding allowed for the identification of dynamic biomarker(s) of response to anti-PD1 in combination with either anti-CD137, anti-CTLA4, anti-CD40, or IL-2. Predicted poor responders identified early after neoadjuvant-treatment could be converted into long-term survivors by further expanding their tumor-specific T cells with a single dose of IL-2 treatment. Translationally, the biomarker(s) identified were validated in PBMCs from human melanoma patients treated with neoadjuvant and adjuvant anti-PD1+anti-CTLA4. Together this work provides compelling support for new-found interest in the development of human neoadjuvant immunotherapy trials.

Keywords: Neoadjuvant immunotherapy, Immunotherapy, Cancer surgery, Immune checkpoint blockade.

## B014 / Whole blood dendritic cell immunoprofiling in advanced melanoma patients and responses to checkpoint inhibitor immunotherapy

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The conventional type 1 dendritic cell subset (cDC1, also known as CD141+ DCs in humans) are essential for the development of protective CD8+ anti-tumor immune responses and efficacy of immunotherapies as demonstrated by animal models. However, very little is known about DC subsets in human cancer patients. We developed a whole blood assay for quantitation, phenotyping, and functional characterization of human DC subsets (CD141+ DCs, CD1c+ DCs, and plasmacytoid DCs (pDCs)) by flow cytometry and compared these in healthy donors and in patients with stage III and IV metastatic melanoma. CD141+ DC and pDC numbers were decreased in stage IV melanoma patients compared to healthy controls. Significantly higher CD141+ and CD1c+ DC numbers were found in male patients, but DC numbers did not appear to correlate with age. We further examined DC function post toll-like receptor (TLR) stimulation by assessing costimulatory molecules CD40 and CD83, and the inhibitory ligand PD-L1. Following stimulation with TLR3 and TLR7/8 agonists, CD141+ DCs in melanoma patients were selectively impaired in their ability to induce CD83. TNFα, IL-1β, and IL-8 were significantly elevated in patient plasma following TLR stimulation compared to equivalently-treated healthy control plasma, whereas IFNα2 was decreased. We further examined patients undergoing anti-PD-1 and/or anti-CTLA-4 immunotherapy by assessing samples pre-treatment, and at 6 weeks-, 12 weeks-, and 6 months during treatment. While DC numbers prior to treatment did not correlate with responses, all DC subsets were decreased by 6 months into treatment selectively in non-responders. CD83 expression on stimulated CD141+ and CD1c+ DCs was also significantly lower in non-responders by 6 months into treatment compared to that of responders. IFNλ1 detected in plasma following TLR3 and TLR7/8 stimulation was significantly higher in responders than in non-responders when

pre-treatment samples were assessed. IL-1β and IFNγ were also higher in responders when 6-month on-treatment samples were assessed. Taken together, these results demonstrate impairments in CD141+ DCs in advanced melanoma patients and provide a rationale for strategies to enhance CD141+ DC numbers and/or function to improve tumor immune responses.

Keywords: Melanoma, Dendritic cell, Immune checkpoint inhibitor, Whole blood.

## B015 / Genetic and pharmacologic suppression of the myeloid-cell specific Src-kinase HCK improves immune checkpoint-mediated anti-tumor responses

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While immunotherapies that target PD1 and CTLA4 have shown impressive clinical success in several cancers such as melanoma and renal-cell carcinoma, many solid malignancies remain refractory to treatment with immune checkpoint inhibitors. One of the underlying reasons for this has been attributed to immunosuppressive tumor microenvironments (TME) characterized by increased infiltration of myeloid cells, including alternatively-activated neutrophils and macrophages. The latter display an immune suppressive and tumor-promoting endotype, which limit the recruitment of effector T cells. Accordingly, strategies are pursued to limit the abundance and recruitment of myeloid cells in to the TME, as well as to interfere with their plasticity and associated polarization towards alternatively activated endotypes.

Elevated expression of the myeloid-specific Src-family kinase Hematopoietic Cell Kinase (HCK) occurs in a majority of solid malignancies and correlates with poor patient survival. Using genetic mouse models of constitutive HCK activation (HCKCA) and HCK-knockout (HCKKO), we have previously shown in adaptive bone marrow chimeras that the HCK genotype affects the growth of endogenous and xenograft tumors [Poh et al, Cancer Cell 2017]. These observations directly correlate with spontaneous HCK-CA-dependent polarization of tumor-associated macrophages to an alternatively-activated Arg2-high/Ym1-high/Tie2-high/NOS-low endotype, which is markedly reduced in HCKKO cells. Importantly, inhibition of HCK signaling with a small molecule kinase inhibitor reduced the growth of endogenous mouse and human xenograft tumors by impairing alternative macrophage polarization in wild-type but not in HCKKO hosts.

Using syngeneic models of gastrointestinal cancer or melanoma, we now demonstrate that the anti-tumor effect of HCK suppression is further enhanced when combined with anti-PD1, anti-CTLA4, or agonistic-CD40 based immune therapy. These observations are underpinned by a mechanism whereby these antibodies confer increased tumor cell apoptosis in HCKKO hosts. We show that this synergy is sensitive to CD8+ T-cell ablation, and we functionally attribute this effect to increased production of cytotoxic IFNgamma, Perforin and Granzyme B by CD8+ T and NK cells, and of IL12 by macrophages. Likewise, pharmacologic inhibition of HCK also augments the anti-tumor activity of anti-PD1, anti-CTLA4, and agonistic-CD40 antibodies by reversing the alternatively-activated macrophage endotype and associated immunosuppressive TME to enable infiltration of effector T-cell in to the tumor cores.

Collectively, our observations establish HCK as a readily targetable myeloid-specific kinase to complement current approach (i.e. inhibition of Csf-1R and/or PI3Kgamma/delta) to reverse the immunosuppressive TME in solid cancers and to improve the efficacy of immunotherapy.

Keywords: Immunotherapy, Solid malignancies, Myeloid cells, Scr-family kinase.

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Poh et al, Cancer Cell 2017

## B016 / In-vitro study of combinatory therapy of the oncolytic adenovirus ONCOS-102, checkpoint inhibitors and standard of care (pemetrexed plus cisplatin) in malignant mesothelioma

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Malignant mesothelioma (MM) is an aggressive and a rare form of cancer that develops from mesothelium. The median survival time for mesothelioma patients after diagnosis is typically only around 12 months. MM tumors are often poorly responsive to standard therapies and incidence is constantly increasing worldwide. The low incidence of MM has led to limited investment in discovery and research of new drugs. There is therefore a high medical need for new treatment modalities.

Oncolytic adenoviruses are promising and potentially powerful immunotherapy tools for treatment of cancer. This approach exploits the high immunogenicity of adenovirus. In addition, oncolysis releases tumor epitopes for processing of antigen presenting cells (APC) and may ultimately lead to the development of adaptive cellular immune responses specific for tumor epitopes. Immunogenicity of adenovirus can be further enhanced by arming the virus with an immune-stimulatory transgene. The overall antitumor efficacy can be potentially enhanced by combining viral immunotherapy with other agents such as check point inhibitors. Indeed, in our previous study we have observed synergistic anti-tumor effect when combining oncolytic adenovirus ONCOS-102 with pembrolizumab in humanized mice melanoma model.

ONCOS-102 is an oncolytic adenovirus armed with human GM-CSF and an Ad5/3 chimeric capsid. It has shown to be well tolerated in phase I study (NCT01598129) wherein it induced antitumor immunity, infiltration of CD8+ T cells to tumors, and up-regulation of PD-L1. In this study, local ONCOS-102 treatment of patients with treatment refractory pleural mesothelioma induced systemic anti-tumor CD8+ T cell responses and tumour infiltration of CD8+ T cells.

The aim of this in-vitro study was to examine the benefits of a combining ONCOS-102 with the immunotherapeutic anti-PD-1 antibody, pembrolizumab and standard of care (SoC/pemetrexed plus cisplatin) which will next be followed by in-vivo experiments. The hypothesis being that co-administration of agents, such as anti-PD1 antibodies and ONCOS-102, may reactivate tumor infiltrating leukocytes against the numerous exposed tumor-associated antigens released by lysis as the final step of viral replication.

Cytotoxicity was investigated with assays for measuring apoptosis, necrosis and cell viability. The amount of apoptosis and necrosis after treatment was assessed by Annexin V and PI staining. Annexin V staining showed that MSTO-211H, NCI-H226 and Mero-95 were sensitive to ONCOS-102 treatment. SoC treatment, used as a positive control, significantly triggered apoptosis and necrosis on all the cell lines tested whereas pembrolizumab had no effect.

Cell viability was investigated by MTS assay. SoC diminished cell viability for MSTO-211H, Mero-82 and Mero-95 cell lines. Treatment with ONCOS-102 (50 and 100 VP/cell) resulted in reduction of cell viability (MTS) for all investigated 5 mesothelioma cell lines.

Calreticulin exposure was triggered after treatment with SoC for all the cell lines but no calreticulin exposure was induced after ONCOS-102 treatment.

HMGB-1 release was induced after treatment with ONCOS-102 for all cell lines except for NCI-H2452.

All cell lines highly expressed PD-L1 (more than 85%) except NCI-H2452, for which only 45% of the cells expressed PD-L1.

Data indicate that NCI-H226 and MSTO-211H cells responded well to ONCOS-102 monotherapy treatment and also to SoC therapy

(pemetrexed plus cisplatin). Additive effect was further observed when ONCOS-102 was combined with SoC. Pembrolizumab alone had no adverse effects on any cell lines tested. However additive effect was observed when ONCOS-102 was combined with pembrolizumab. These findings give a rationale for the further preclinical testing of ONCOS-102 in combination with first-line chemotherapy and pembrolizumab in malignant mesothelioma.

Keywords: oncolytic adenovirus, ONCOS-102, melanoma, CPI.

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## B017 / Tumor-intrinsic factors that prevent leukocyte recruitment in a spontaneous mouse model of gastric cancer

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Gastric cancer is the sixth most frequent type of cancer worldwide and the third leading cause of cancer-related death. The success of immunotherapy makes it an appealing strategy to treat gastric cancer, but recent clinical trials have reported limited efficacy of immune checkpoint inhibitors in this indication. Developing new therapeutic strategies is thus essential to improve the efficacy of immunotherapy and the outcome in this disease. Here we characterized the anti-tumor immune response in a mouse model of spontaneous gastric cancer, the CEA424-SV40 T Ag mice. These mice develop stomach tumors that grow as multifocal plaques in the gastric mucosa and progressively invade the surrounding tissues. We observed an age-dependent increase of leukocyte numbers in the tumors, consisting mainly of PD-L1+ myeloid cells and B cells. Low numbers of CD62L- CD44+ effector T cells were also detected, expressing markers of T-cell activation as well as immune checkpoint molecules commonly associated with T-cell exhaustion such as PD-1. Detailed histological analysis revealed that the leukocytes were largely excluded from the tumor and were confined to the tumor-surrounding stroma, in a pattern characteristic of immune-excluded cancer. As reported in literature, immune-excluded tumors are less likely to respond to immune checkpoint blockade, and in line with this, we observed no efficacy of anti-PD-1 therapy. RNAseq analysis of the tumor revealed that the TGFβ signalling pathway together with factors typical of stroma remodelling were upregulated in late stage tumors in these mice. Using the RNAseq data from these mice, we developed a new gene signature for stroma remodelling. Importantly, this signature can be translated to the human system and serves as a better predictor for overall survival in patients with stomach adenocarcinoma than previously described signatures. Our findings thus suggest that the CEA424-SV40 T Ag mouse model mirrors essential pathological immune mechanisms present in gastric cancer patients with poor prognosis. We propose that these mice are a good platform to test new therapeutic strategies, including combinatorial treatment with immune checkpoint blockade in order to enhance the outcome of immunotherapy in gastric cancer

Keywords: Gastric cancer, immune-excluded tumors, Stroma remodeling, TGFbeta.

References:
Bourquin et al. 2010 Hegde, Karanikas, and Evers 2016 Zeng et al. 2019

## B018 / CD40 agonist primes the T cell response to αPD1 treatment in an αPD1 resistant model of muscle-invasive bladder cancer

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Bladder cancer (BCa) represents the fourth most common cancer in men, with poor patient prognosis for advanced disease [1]. The recently introduced immunotherapy have allowed to improve the treatment of advanced BCa patients, but only a fraction of them respond to immune checkpoint blockade (αPD1/αPDL1) [2]. To better understand the immune biology of the disease and its relevance for the patient's outcome, we developed a genetic mouse model for BCa that recapitulates the various stages of the human disease. Specific deletion of Tp53 and Pten in the bladder induced non-muscle invasive BCa, followed by transition to muscle invasive BCa (MIBC) and then metastatic disease. With tumor progression, we observed a transition from an inflammatory microenvironment to a pro-tumoral/immunossupressive one, going along with a M1 to M2 transition of macrophages, increased expression of PD1 by T cells and accumulation of regulatory T cells. Interestingly, this MIBC model is resistant to αPD1 treatment. After investigating various immunotherapy combinations, we found that αCD40+αPD1 led to an increase of CD8 TIL infiltration and IFNγ production, a re-polarization of macrophages toward a M1-like phenotype and significantly prolonged mouse survival. We revealed that CD8 T cells, macrophages and IFNγ were necessary for the control of the tumor, and that IFNγ production by CD8 T cells was directly related to the repolarization of macrophages. To conclude, our mouse model is representative of the large proportion of MIBC patients that does not respond to αPD1 treatment, making it suitable to test new immunotherapeutic strategies for these patients. Treatment with αCD40+αPD1 was successful by reprograming the immune microenvironment, suggesting its potential as an effective combinatorial therapy for patients with MIBC that are resistant to currently available therapies.

Keywords: Bladder cancer, anti-PD1, anti-CD40.

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## B019 / Improving the resistance against PD-1 blockade therapy in melanoma lung metastasis by STING agonist-loaded lipid nanoparticles

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The appearance of immune-checkpoint inhibitors such as the programmed cell death 1 (PD-1) antibody has revolutionized the field of cancer therapy. However, positive responses were observed in only a minority of patients treated. In some types of cancers that are resistant to PD-1 blockade therapy, a weak immune response and a few immune cells were observed in the tumor microenvironment, which is referred to as a cold tumor. In this situation, activating the immune system in our body is a promising strategy for converting cold tumors into hot tumors. Cyclic di-GMP, a cyclic dinucleotide found in bacteria, is known to induce an immune response by activating the stimulator of interferon genes (STING)

pathway. The STING pathway is an innate immune pathway sensing cytosolic DNA and an agonist of the STING pathway would be a promising adjuvant for use in cancer immunotherapy. We reported on the development of a cyclic di-GMP (for use as a STING agonist) loaded in a lipid nanoparticle (STING-LNP), which showed strong antitumor immune responses (ref. 1 and 2). In this study, we report on an evaluation of the potential of the STING-LNP to convert cold tumors into hot tumors, in which an immune response is actively generated and the resistance against PD-1 blockade therapy is improved.

Evaluations of antitumor activity were performed by measuring the expression of luciferase in a lung metastasis mouse model prepared by the administration of B16-F10-luc2 cells via the tail vein. The administration of the PD-1 antibody alone showed no antitumor activity, indicating its resistance against PD-1 blockade therapy. On the other hand, the use of a combination of the STING-LNP and the PD-1 antibody showed a remarkable antitumor effect, suggesting that the resistance against PD-1 blockade therapy was improved by the treatment with the STING-LNP. Subsequently, we elucidated the mechanism of the reduced resistance to PD-1 blockade therapy by the treatment of STING-LNP. An analysis of gene expression in a lung with B16-F10-luc2 metastasis indicated that NK cells, helper T cells, PD-1 expression and IFN-γ production were all increased by the STING-LNP treatment, suggesting that the cold tumor was converted into a hot tumor. Moreover, the expression of the programmed cell death ligand 1 (PD-L1) was strongly enhanced in the tumor colony in the lung tissue after the STING-LNP treatment. These results indicate that the conversion to a hot tumor by the STING-LNP administration induced the expression of PD-L1 on the cancer cells and established immune suppression via PD-1/PD-L1 interaction, resulting in the appearance of the PD-1 blockade effect.

Collectively, the STING-LNP represents an example of a promising adjuvant system for improving the resistance against PD-1 blokade therapy in melanoma lung metastasis and that a combination therapy using the STING-LNP and a PD-1 antibody can be an effective cancer immunotherapy.

Keywords: PD-1 blockade, STING agonist, delivery system, adjuvant

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## B020 / Methionine increases the efficacy of suboptimal anti-PD-1 treatment by boosting an anti-tumor γδ/ CD8+ T cell crosstalk

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Tumors develop potent mechanisms to evade immune surveillance, such as T cell inhibition through the PD-1/ PD-L1 immune checkpoint pathway. This led to the development of blocking monoclonal antibodies that produced remarkable success in the treatment of some advanced-stage cancers, particularly in melanoma and lung carcinoma. Regrettably, most patients in many cancer types do not respond or have limited clinical benefit from anti-PD-1 antibody therapy, thus urging the need to improve its efficacy. Based on the overt negative impact of oxidative stress on T cell responses, we explored here the potential of combining checkpoint inhibition with the administration of methionine, whose metabolism provides substrates for producing the key cellular anti-oxidant, glutathione. We found that methionine supplementation enhances the efficacy of suboptimal anti-PD-1 therapy in an orthotopic murine model of triple-negative breast cancer. In comParison with anti-PD-1 single therapy, the combination with methionine supplementation in vivo leads to increased accumulation of γδ T cells and IFN-γ-producing CD8+ T cells with enhanced killing ability in the tumor bed. Depleting strategies and genetic approaches revealed that both CD8+ and γδ T cells are necessary for the decreased tumor growth upon combination treatment. Interestingly, γδ T cells appear to be upstream of the CD8+ T cell response, since the accumulation of IFN-γ-producing CD8+ T cells within tumor lesions is markedly reduced in TCRδ-deficient mice. These data reveal an unanticipated γδ / CD8+ T cell axis, specifically boosted by methionine metabolism, that enhances the efficacy of suboptimal anti-PD-1 therapy in pre-clinical models of breast cancer.

Keywords: Anti-PD-1, methionine, gamma-delta T cells, CD8 T cells.

## B021 / Autophagy inhibits abscopal responses to radiation driven by mitochondrial DNA

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Background: Macroautophagy (autophagy) is an evolutionarily conserved cellular mechanism culminating with the lysosomal degradation of dispensable, damaged or potentially toxic cytoplasmic structures including permeabilized mitochondria. Autophagy helps cancer cells to adapt to harsh environmental conditions and to resist therapy. However, autophagy in malignant cells is also key for anticancer immune responses. Thus, whether autophagy should be inhibited or activated in the context of cancer therapy remains debated. Since: (1) autophagy has been shown to play a key role in the removal of multiple sources of cytosolic DNA including micronuclei and permeabilized mitochondria, (2) cytosolic DNA leads to type I interferon (IFN) secretion, and (3) type I IFN is required for systemic immune responses activated by radiation therapy (RT), we asked the question as to whether selectively inhibiting autophagy in cancer cells may boost the ability of RT to initiate anticancer immunity. Moreover, we evaluated the contribution of mitochondrial DNA (mtDNA) to abscopal responses driven by RT.

Methods/Tools: CRISPR/Cas9 technology was used to render mouse mammary carcinoma TSA cells autophagy deficient, and chemical inhibitors of autophagy were employed. Autophagy-competent versus –deficient cells were characterized for autophagic proficiency (by immunoblotting), resistance to cell death induced by RT (by multicolor flow cytometry and clonogenic assays), sensitivity to RT in vivo, in immunocompetent syngeneic hosts (by tumor growth tests), and production of type I IFN (by RT-PCR and ELISA). Alongside, mitochondria-deprived (Rho0) TSA cells were generated and tested for accumulation of cytosolic DNA (by immunofluorescence and subcellular fractionation), and type I IFN expression (by RT-PCR) upon irradiation. Moreover, TSA cells were employed to generate slightly asynchronous tumors in immunocompetent syngeneic mice. Only one of these tumors (that was either autophagy-competent or-deficient) was irradiated in the context of CTLA4 inhibition, and the response of both the irradiated and non-irradiated (abscopal) tumor was monitored.

Results: Autophagy inhibition increased the sensitivity of mouse mammary carcinoma cells to RT, in vitro and in vivo. The ability of RT to drive cytosolic DNA accumulation and type I IFN production was lost in Rho0 cells, and cytosolic DNA was found in the proximity of mitochondria (but not nuclei) in wild-type cells. Moreover, pharmacological and genetic autophagy inhibition increased the capacity of mouse mammary carcinoma cells to secrete type I IFN in response to RT, correlating with increased accumulation of cytosolic DNA. Finally, immunocompetent mice bearing syngeneic autophagy-deficient mouse mammary carcinoma cells mounted improved abscopal responses to RT (in the context of CTLA4 blockade) as compared to immunocompetent mice bearing syngeneic autophagy-competent cells, as determined by growth in-

hibition of a distant, non-irradiated, autophagy-competent lesion.

Conclusion: Autophagy inhibits abscopal responses driven by RT as a consequence of its ability to dispose of permeabilized mitochondria, which stands out as a major initiator of type I IFN secretion in this setting.

Keywords: systemic anti-tumor immunity, Type I IFN, CTLA-4.

## B022 / Sustained compensatory p38 MAPK signaling activation following RAS/RAF/MEK inhibition induces upregulation of the immunosuppressive protein CD73

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The RAS/RAF/MEK (MAPK) pathway play a central role in immune regulation within the tumor microenvironment and intriguingly inhibitors of this pathway have been shown to both suppress and promote tumor-immune activity suggesting that the immunomodulatory activity of MAPK inhibitors is multifaceted and complex. CD73 has recently attracted substantially attention due to its immunosuppressive function through adenosine production. The expression of CD73 has been linked to inflammation mediated MAPK signaling, through ERK1/2 and c-jun, and shown to be altered by inhibition of this pathway.

Here we show that low CD73 expression significantly correlates with an improved response to cetuximab in colorectal cancer patients, thus highlighting the importance of CD73 in treatment targeting the MAPK pathway. While we confirmed previous reports showing that CD73 expression is downregulated in some patient tumors during BRAF inhibition, our clinical data including head and neck squamous cell carcinoma and melanoma patients, indicates that in the majority of patients CD73 expression was either unchanged or upregulated. Interestingly, other transcriptional targets of the MAPK pathway, including cyclin D1 expression, was downregulated in the majority of the latter patient group suggesting that the transcriptional activity was indeed inhibited in these patients. We further investigated the effect of inhibition of the MAPK pathway on human and mouse cell lines as well as PDX models. We found that while MAPKi initially significantly decreased CD73 expression in most cell lines, CD73 was subsequently re-expressed and significantly upregulated compared to the initial CD73 level prior to treatment in a subset of the cell lines tested. This MAPKi-induced CD73 expression was mediated by sustained cross-activation of the p38 pathway and furthermore pharmacological inhibition of p38 diminished the upregulation of CD73. This pattern of CD73 regulation was also observed in clinically relevant pancreatic and breast cancer PDX models.

Our results highlight the unfavorable effect of CD73 expression during treatment targeting the MAPK pathway and that compensatory signaling pathways is activated following MAPK inhibition, which might lead to increased CD73 expression in a subset of tumors. As a consequence, this might contribute to the development of immunologic resistance and emphasizes the importance of co-targeting CD73 in order to enhance the anti-tumor effect of MAPK targeting drugs.

Keywords: CD73, MAPK.

## B023 / Functional impact of anti-PD-L1 treatment on specific lung (tumor) residing myeloid cell subsets

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Antibodies targeting Programmed Death-1 (PD-1) or its ligand became a first-line treatment option for metastatic non-small cell lung cancer (NSCLC) patients. Unfortunately, about 75% of patients don't show any benefit1. The main clinically applied biomarker is PD-L1 expression on tumor cells. However, preclinical observations reveal that not PD-L1 on tumor cells, but on tumor infiltrating myeloid cells represents a major determinant for therapy outcome2, 3. Further, the lung represents an immunologic organ packed with different innate myeloid subsets that could interact with anti-PD-(L)1 antibody as they express both Fc receptors and/or PD-(L)1. Despite these observations, the functional impact of anti-PD-L1 therapy on myeloid cells within healthy and NSCLC tissue as well as systemically remains largely unknown.

To evaluate the abundance of specific PD-(L)1+ myeloid subsets during NSCLC progression, C57BL/6 mice were intravenously challenged with Firefly luciferase (Fluc) encoding Lewis Lung Carcinoma (LLC) cells. Next, mice were treated every three days with anti-PD-L1 or isotype control for four times in total. NSCLC engraftment and PD-(L)1 expression was evaluated on weeks 1, 2 and 3 using immunohistochemistry and flow cytometry. In addition, sorted myeloid subsets were functionally evaluated using an ex vivo 3D spheroid killing assay next to qPCR for alternative checkpoints, arginase 1, nitric and super oxide evaluation.

First, only a marginal therapeutic benefit without increase in CD8+ T cell infiltration was observed upon anti-PD-L1 therapy. In contrast, particular myeloid subsets decreased significantly within NSCLC bearing lungs. More specifically the fraction of Ly6G+ neutrophils decreased during anti-PD-L1 therapy while the fraction of MHCIIlo F4/80+ macrophages ('M2'), Ly6C+ inflammatory (IM) and Ly6C- residential monocytes (RM) only decreased after therapy. Systemically, the ratio of MHCIIlo/MHCIIhi macrophages and the amount of CX3CR1+ patrolling monocytes decreased in bone marrow or/and in spleen respectively. In contrast, PD-L1+ neutrophils and macrophages decreased in blood and spleen while all PD-L1+ monocytic fractions decreased only in spleen. When lung-residing macrophages and monocytes were sorted from healthy or tumor-bearing mice, we could show that in vivo anti-PD-L1 treatment increased: 1) SIRP𝛼 expression on the macrophages and IMs, 2) arginase 1 expression in the RMs, 3) superoxide production in the 'M2' macrophages, and 4) the CD8+ T-cell stimulating potential in an ex vivo 3D spheroid killing assay specifically for the 'M1' macrophages and RMs.

These findings shed light on our relatively confined knowledge of myeloid cell related responses to anti-PD-L1 treatment that could provide novel rationales for potent combination therapies.

Keywords: anti-PD-L1 therapy, lung cancer, myeloid cells, spheroids.

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## B024 / PI3Kαδ inhibitor and radiation enhance anti-tumor effect of PD-1 blockade in syngeneic tumor model

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Background: Breast cancer has been showing relatively poor response to immune checkpoint blockades. We hypothesize that in situ tumor vaccination via radiation therapy (RT) and suppression of immune tolerance via PI3Kδ inhibition could enhance the efficacy of immune checkpoint blockade and evaluated whether RT and a novel PI3Kαδ inhibitor could enhance the efficacy of a PD-1 blockade in immune-competent syngenic triple negative breast cancer model.

Methods: 4T1 murine breast cancer cells were grown subcutaneously in the hind limb of BALB/c mice. Tumors were irradiated using 24 Gy/3 fractions. PD-1 blockade and PI3Kαδ inhibitor were administered every other day for two weeks, respectively. Tumor size was measured periodically to evaluate efficacy of each modality and combination therapy. Immune-modulatory function was evaluated using FACS, Elispot assay and immunohistochemical staining. Results: Triple combination of RT, PD-1 blockade, and PI3Kαδ inhibitor significantly delayed tumor regrowth whereas PD-1 inhibitor alone showed only modest effect in 4T1 syngenic TNBC model. FACS and IHC study for immune repertoire using tumor samples showed that RT and PD-1 blockade modestly in-

creased the proportion of cytotoxic CD8+ T cells and PI3Kαδ inhibitor led to decrease the proportion of regulatory T cells and MDSCs. Triple combination showed remarkable increase of cytotoxic CD8+ T cells suggesting synergistic immune modulatory effect of RT, PD-1 blockade and PI3Kαδ inhibitor. Triple combination led to significant upregulation of c-GAS/STING pathway in the tumor and increased IFN-γ level in blood as well compared to each modality alone.Abscopal effect was observed and it correlated with the accumulation of CD8+ T cells in the spleen.

Conclusion: Taken together, combination of RT and PI3Kαδ inhibitor significantly enhanced immune stimulatory function in immune competent syngenic TNBC model and enhanced the response of PD-1 blockade via non-redundant synergistic immune modulatory effect. This study provides a preclinical rationale for the combination of PI3Kαδ inhibitor and RT with PD-1 blockade to overcome the immune tolerance of breast cancer. (Work supported by the grants from NRF #2017R1A2B4002710 & #2017M2A2A7A01018438 to In Ah Kim)

Keywords: PI3Kαδ, Radiation, PD-1.

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## B025 / Intratumoral CD40 agonist (APX005M) in combination with pembrolizumab induces broad innate and adaptive immune activation in local and distant tumors in CPI treatment-naïve metastatic melanoma

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The use of immune-checkpoint inhibitors (CPI) has become an important modality in the treatment of metastatic melanoma (MM). However, long-term survival and durable remission rates remain low and new treatment options are needed to improve clinical outcomes. CD40 activation on antigen presenting cells (APCs) initiates their ability to prime and activate CD8+ T cells. APX005M is a humanized IgG1 CD40 agonistic antibody that binds with high affinity to human CD40 expressed on APCs. Our pre-clinical studies have demonstrated that intratumoral CD40 activation induced systemic anti-tumor effects and synergize with anti-PD-1 therapy. Therefore, we hypothesized that intratumoral administration of APX005M in a single lesion in combination with pembrolizumab would stimulate local APCs and potentiate CD8+ T cell-mediated local and abscopal anti-tumor responses. This is an ongoing dose I/II study of image guided intratumoral (IT) APX005M in combination with systemic pembrolizumab in MM. Approximately 41 participants will be enrolled, all patients (pts) will receive IT APX005M every 3 weeks for a total of 4 doses. The dose escalation portion of the trial has been completed with 18 pts enrolled in 5 dosing cohorts of APX005M at 0.1, 0.5, 1, 3 and 10 mg in combination with standard pembrolizumab at 2 mg/kg. Biomarker analyses of blood and tumor biopsies are being performed to measure immune activation using immunophenotyping including mass cytometry (CyTOF), multiplex immunofluorescence, TCR sequencing and gene expression analyses. The combination of APX005M with pembrolizumab did not induce dose limiting toxicity at any dose level of APX005M. Most treatment-related adverse events (TRAEs) were injection-site reactions. No grade ≥3 TRAEs were reported. 13 pts treated across all five dosing cohorts have had disease evaluations (as of May 2, 2019 data cut); 7 pts (54%) achieved partial responses (PR) including 2 pts who received 1 mg of APX005M, 2 pts who received 3 mg of APX005M and 3 pts who received 10 mg of APX005M. Interestingly, partial responses were observed in both PD-L1 negative and positive tumors. 4/13 (31%) pts had stable disease (SD), and 3/13 (23%) pts had progression (PD). Preliminary RNA profiling of tumor biopsies obtained from 3 responding pts treated with APX005M at dose between 3 to 10 mg and 3 nonresponding pts treated with APX005M at lower dose (0.1 and 1 mg) revealed an induction of broad innate and adaptive immune activation in the responding pts only. 6 weeks post injection biopsies obtained from 2 responding pts in both injected and un-injected tumors showed upregulation of genes expressed in a variety of APCs including DCs, macrophages, and B cells (ITGAX, CD68, CD79A) as well as upregulation of genes associated with their activation (CD80, CD86, and HLADRA). There were also increase in the genes associated with T-cell infiltration and effector functions (CD3G, CD3D, CD8A, IFNG, and GZMB). Consistently, TCR sequencing analysis demonstrated an increase in T cell infiltration and clonality post treatment only in the responding patient reflecting the induction of a more oligoclonal T cell repertoire. Strikingly, the increase in T-cell infiltration and clonality was observed in both injected and un-injected lesions. Collectively, results from the dose escalation portion of this trial demonstrated that combination of pembrolizumab with intratumoral APX005M is well tolerated, has a notable clinical response accompanied by broad innate and adaptive immune activation in both local and distant lesions. Updated biomarker and clinical response data will be presented.

Keywords: Intratumoral CD40 agonist (APX005M) in combination with pembrolizumab, Metastatic melanoma, innate and adaptive immune activation.

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## B026 / A novel bifunctional anti-PD-1 / IL-7 fusion protein potentiates effector function of exhausted T cell and disarms Treg suppressive activity

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Introduction Despite the clinical success of checkpoint blockade therapies over other cancer treatments, most patients remain unresponsive or fail to develop a durable response after PD-(L)1 therapy. We explored a novel combination therapy and designed a second generation of PD-1 antibody by fusing IL-7 cytokine to the Fc portion. IL-7 is an optimal target for immunotherapy to preferentially stimulate effector T cell (Teff) functions over regulatory T cells (Treg) due to the differential expression of IL-7R and poor capacity of IL-7 to stimulate Treg proliferation (1). Moreover, it has been recently published that PD-1 blockades improve IL-7 signaling in exhausted T cells (2) rationalizing our combination approach anti PD-1 antibody + IL-7. We hypothesized that our bispecific anti PD-1/IL-7 fusion molecule will outperform anti PD-1 therapy by promoting a sustaining effector anti-tumor response and by impeding the immunosuppressive activity of Treg. Experimental procedure Proliferation (H3 thymidine), IFN-γ secretion, IL-7R signaling (pSTAT5) and NFAT activation (PD-1 bioassay, PromegaTM) assays were tested to determine the efficacy of the anti PD-1/IL-7 on naïve and exhausted-like T cell population in vitro. For the suppressive assay, CD4 Treg and autologous CD8 Teff were co-cultured (1:1) with IL-7, anti PD-1/IL-7, IL-2 or IL-15. Teff and Treg proliferation were assessed on 5 days using cell tracer. In vivo tumor mouse models were developed by orthotopic injection of hepatocarcinoma cells (Hepa1.6), lung carcinoma cells (LLC-1) or mesothelioma cells (AK7).

Summary of data Our anti PD-1/IL-7 bispecific antibody efficiently blocks the PD-1/PD-L1 and PD-L2 interactions and the PD-1 mediated inhibitory signal (pSHP1). Importantly, we observed that the IL-7 portion synergizes with the anti-PD-1 to enhance TCR mediated signaling (NFAT). Using a model of repeated TCR stimulation in vitro, we recapitulated chronic antigen stimulation and characterized the capacity of T cells to respond to IL-7. Although IL-7R expression decrease overstimulation while PD-1 expression increased, we observed that partially and fully exhausted T cells still efficiently respond to IL-7 as shown by pSTAT5 activation. Moreover, IL-7 was able to maintain proliferation of partially and fully-exhausted T cells.

Knowing that Tregs have a key suppressive function in the tumor microenvironment, we explored the possibility that anti PD-1/IL-7 affect Treg functions. In a Treg/Teff coculture assay, we observed that IL-7 and the anti PD-1/IL-7 fusion protein does not stimulate regulatory T cells (Treg) proliferation, in contrast to IL-2 and IL-15 cytokines. We also demonstrated that IL-7 as the anti PD-1/IL-7 abrogates the capacity of Treg to inhibit proliferation and IFN-g secretion of effector CD8+ T cells.

To investigate the potential therapeutic effect of anti PD-1/IL-7 in vivo, we first developed and characterized multiple orthotopic tumor mouse models that are sensitive and resistant to anti PD-1 treatment. In PD-1 sensitive model (Mesothelioma), only 10% of intratumoral T cells express IL-7R whereas in PD-1 resistant model (Hepatocarcinoma and Lung carcinoma), 40-60% of Tumor-infiltrating T cells (both CD4 and CD8) express IL-7R and are sensitive to IL-7 stimulation ex vivo (pSTAT5 activation). Experiments are ongoing to evaluate the efficacy of Anti PD-1/IL-7 versus anti PD-1 alone in these tumor models. Our preliminary data showed that the anti PD-1/IL-7 bispecific can reactivate intratumoral T cells that are resistant to PD-1 therapy.

Conclusion Our data validate the therapeutic potential of anti PD-1/IL-7 molecule. The bifunctional anti-PD1/IL-7 favors the T cell effector over T regulatory immune balance by stimulating effector T-cell functions (proliferation, IFN-γ) while disarming regulatory T cells.

Keywords: bispecific antibody, PD-1, IL-7, exhaustion.

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## B027 / MGD019, a PD-1 x CTLA-4 Tetravalent Bispecific DART® Protein, Provides Optimal Dual Checkpoint Blockade

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Combinatorial blockade of PD-1 and CTLA-4 has shown clinical benefit beyond that observed with individual mAbs, albeit with increased toxicity. Co-expression of the checkpoint receptors creates an opportunity for selective, enhanced blockade of dual PD-1/CTLA4 expressing cells through use of a bispecific inhibitor targeting both pathways simultaneously.

Consistent with previous reports, multiplex in-situ hybridization (ISH) demonstrated enrichment of PD-1/CTLA-dual positive cells in neoplastic lesions relative to normal tissues, where distinct populations expressed CTLA-4 or PD-1. PD-1 x CTLA-4 molecules were designed as either bivalent or tetravalent bispecific engagers with a human IgG4 backbone; in vitro characterization revealed that both 1 x 1 and 2 x 2 bispecific PD-1 x CTLA-4 formats block each checkpoint molecules, with enhanced blockade of CTLA-4 over that achieved with anti-CTLA4 mAb on dual expressing cells. As expected, 1 x 1 format molecules are less efficient in blocking PD-1 in cell models lacking CTLA-4 expression, while 2 x 2 molecules demonstrate full PD-1 blockade equipotent to a nivolumab replica. Likewise, the 2 x 2 format molecule effectively blocks CTLA-4 ligand interactions on PD-1negCTLA4pos cells, while the 1 x 1 design is significantly less efficient. The tetravalent bispecific molecule was designated as MGD019 and selected for clinical development.

In primary cell assays in which both CTLA-4 and PD-1 contribute to inhibit T-cell activation, MGD019 enhances antigen-driven in vitro T-cell activation to a level comparable to the combinatorial PD-1 plus CTLA-4 blockade. Tumor microenvironment models that recapitulate vascular or stromal compartments confirmed MGD019 induces in vitro immune response profiles comparable to those observed with replicas of ipilimumab plus nivolumab. Unlike ipilimumab, however, MGD019 does not reduce the number of peripheral blood Tregs ex vivo.

MGD019 was well tolerated in cynomolgus monkeys, with no mortality or significant adverse findings up to 100 mg/kg QWx4. T-cell proliferation in the periphery and expansion in lymphoid organs was observed, with increases in ICOS+ CD4 cells and memory T cells, findings attributable to the CTLA-4 blocking arm, since the anti-PD-1 mAb precursor was devoid of these activities.

In summary, MGD019 offers the convenience of a single molecule administration for dual checkpoint blockade. In addition to providing full blockade on cells expressing PD-1 or CTLA-4 individually, MGD019 exploits dual target avidity resulting in preferential engagement and enhanced blockade on cells that express both checkpoint molecules, a feature that could provide additional benefits given the preeminent co-expression of CTLA-4 and PD-1 by TILs. These data indicate support clinical testing of MGD019 in

cancer patients.

Keywords : PD-1, CTLA-4, bispecific, memory.

## B028 / Tumor-expressed B7x promotes the induction of regulatory T-cells and resistance to immune checkpoint blockade

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The B7/CD28 families of ligands/receptors play crucial roles in cancer progression, as tumors rely on inhibitory pathways, i.e. immune checkpoints, to evade immune-mediated killing. Blockade of checkpoints such as CTLA-4, PD-1, and PD-L1 is effective in the treatment of many cancers, but resistance to these therapies is common. B7x (B7-H4) is a less characterized member of the B7 family that is frequently overexpressed by human cancers and inhibits anti-tumor immunity. Since B7x mediates an inhibitory pathway that suppresses the anti-tumor immune response, we hypothesized that expression of B7x on tumor cells would reduce the therapeutic efficacy of checkpoint inhibitors such as anti-CTLA-4.

To investigate this potential mechanism of resistance, we used the MC38 syngeneic murine tumor model. Mice were engrafted with MC38 colon carcinoma cells that were either stably transduced to express B7x (MC38-B7x) or were mock transduced (MC38-control). We observed that anti-CTLA-4 treatment was less effective in inhibiting the growth of MC38-B7x tumors as compared to MC38-control tumors. Analysis of tumor-infiltrating immune cells showed that B7x-expressing tumors had increased regulatory T cell (Treg) populations and reduced activation of effector T and NK cells after anti-CTLA-4 treatment. Moreover, increased Tregs were also observed in B7x-expressing tumors of untreated mice. In vitro, we observed that naïve CD4 T cells converted into Tregs at greater rates in the presence of recombinant B7x-Ig protein or B7x-expressing MC38 cells, than with control Ig-protein or B7x-negative cells. When Tregs were transiently depleted in tumor-bearing mice, the resistance of MC38-B7x tumors to anti-CTLA-4 was eliminated, demonstrating that Tregs are necessary for the resistance phenotype. Further, combination treatment with an anti-B7x monoclonal antibody together with anti-CTLA-4 demonstrated synergistic efficacy against MC38-B7x tumors and abolished their resistance to anti-CTLA-4 therapy.

These findings reveal novel roles for tumor-expressed B7x in promoting Treg induction and reducing the efficacy of anti-CTLA-4. Further, we demonstrate the synergistic therapeutic effect of anti-B7x with anti-CTLA-4, highlighting a promising new strategy for cancer immunotherapy.

Keywords : B7x, Resistance, Treg, Checkpoint blockade.

## B029 / Heterogeneity of T-cell receptor and tumor neo-epitope repertoires in experimental glioma in the context of immune checkpoint blockade

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Although immune checkpoint blockade (ICB) therapy plays an increasingly important role in treatment of various tumor entities, gliomas seem resistant to this line of therapy. Recent evidence from a randomized clinical trial did not show a therapeutic benefit in an unselected population of patients with recurrent glioblastoma. However, the blood brain barrier does not seem to be a hurdle in transducing an effective peripheral immune response into tumors treated with ICB, as shown by responses seen in selected glioma patients as well as patients with brain metastases.

This project investigates the mechanisms of effective immune response to ICB targeting CTLA-4 and PD-1 in an experimental syngeneic glioma model, where we found a remarkably clear dichotomy and acquired immune heterogeneity between responder and non-responder mice. Response to ICB was driven by increased numbers of cytotoxic tumor-infiltrating T cells (TILs) and distinct phenotypic changes of TILs. Using state-of-the-art single cell RNASequencing as well as VDJ analysis of TILs from responding and non-responding mice, we were able to faithfully map TIL phenotypes and discover unique T-cell receptor (TCR) signatures. Within our dataset we found 25 distinct clusters of TCRs which are differentially expressed in responding and non-responding mice. Likewise, we were able to classify shared TCR motifs in ICB responding mice, suggestive of a common tumor-antigen driving the expansion of reactive clones in responding mice. Acquired immune heterogeneity was further indicated by distinct tumor neo-epitope repertoires of responder and non-responder tumors, where we identified neo-epitopes that were mainly enriched in non-responder tumors and thus might have been effectively targeted in ICB responders. Moreover, we demonstrated that peptide vaccination in mice with one of our MHC class II neo-epitopes was able to induce a strong and consistent immune response. Collectively, we provided evidence for shared TCR signatures as well as putative tumor neo-antigens associated with effective ICB therapy. These findings might not only be exploited for immunotherapy target discovery in glioma patients but also further broaden our understanding of T-cell therapy in glioma.

Keywords : Immune Checkpoint Blockers, T-cell therapy, Single Cell Sequencing, Glioma.

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## B030 / Playing hide and seek with glioblastoma : Using epigenetic modulators to increase cancer testis and neoantigen expression

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Background Glioblastoma (GBM) is the most common and malignant primary brain tumour in adults. With full treatment the median survival for such patients is still only 14- 24 months (1). The relentless and inevitable progression of glioblastoma is thought to be facilitated in part by an immunosuppressive microenvironment, which weakens the ability of the central nervous system

to mount an effective and tumour-eradicating response. However, despite numerous trials, clinical response in GBM patients to these immunomodulatory drugs, including checkpoint inhibitors, is modest (2-4). This is in part due to the low number of mutations seen in GBM (5). Epigenetic regulation of tumour cells is becoming increasingly recognised as an important factor in tumour immune escape with down-regulation of chromatin modifying genes being shown to lead to increased sensitivity to checkpoint blockade (6) and increased immune-mediated cell killing through increased expression of interferon stimulated genes (7). Here, we examine the effect of a decitabine (DAC), a DNA methyl-transferase inhibitor, on the expression of both cancer testis antigens (CTA) and neoantigens (NAg).

Methods Primary GBM cell lines were created through single cell suspension of fresh tumour specimens taken at the time of surgery and culturing on laminin coated plates using defined neural stem cell media. Neoantigens from Primary and U87MG cell lines were predicted using whole exome sequencing data. Differential expression of CTA and NAg caused by DAC treatment was determined using RNA sequencing data from the cell lines in the presence or absence of 1uM DAC. Peptide specific T cells were isolated from peripheral blood mononuclear cells (PBMC) of autologous donors and from healthy donors, using fluorescence labelled peptide-MHC class I tetramers. T cell functionality was tested through intracellular cytokine staining and/or LDH release killing assay.

results Four out of nine potential neoantigen encoding mutations were significantly upregulated following DAC treatment in U87MG cell line and further 7 in the 4 primary patient cell lines. In addition, a wide range of CTA were consistently upregulated across the 4 primary patient cell lines. We were able to establish 1 NAg specific T cell clone from 1 patient's PBMC and 35 clones responsive to 37/160 peptides across all the cell lines. All T cell clones respond to their specific peptide in a concentration specific manner. Within these, there are T cell clones that are responsive to NAgs that are expressed in the cell line at baseline. These T cell clones are able to recognise and kill the specific cell lines in a TCR-MHC dependent fashion. In addition, we show that T cells specific to NAgs upregulated by DAC show increased activation and killing against cell lines treated with DAC.

Conclusion Here we show for the first time that a large spectrum of CTAs as well as several NAgs are upregulated following DAC treatment. We have optimised a protocol for isolation of a panel of peptide specific T cells, both from patients and healthy donors, for interrogation of increased NAg specific killing following treatment with DAC. Finally, we show these T cells are capable of recognition of the tumours in a NAg specific fashion and increased expression of NAg leads to increased T cell mediated killing.

Keywords: Decitabine, Glioblastoma, Neoantigen, checkpoint inhibitor.

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## B031 / Targeting lysyl oxidase (LOX) favors T cell migration in the tumor stroma and enhances anti-PD1 treatment.

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Cancer immunotherapy is a promising therapeutic intervention. However, complete and durable responses are only seen in a fraction of cancer patients. One of the determinants in the success of T cell-based immunotherapies lies in the ability of effector T cells to reach tumor cells. Solid tumors are characterized by an aberrant organization of the extracellular matrix (ECM) in the form of highly reticulated and long linear collagen fibers, which have been shown to limit T cells infiltration into tumor cell islets. Currently, there are several strategies in development to target the tumor ECM including the inhibition of lysyl oxidase, an extracellular copper-dependent enzyme upregulated in many tumors that catalyzes the cross-linking of collagen. Here, using several relevant preclinical mouse models of pancreatic, breast and bile duct carcinomas combined with dynamic imaging on fresh tumor slices, we investigated the consequences of LOX pharmacological inhibition with beta-aminopropionitrile (BAPN) on the intratumoral migration of T lymphocytes. Our data indicate that treatment of mice with BAPN lead to a significant decrease of tumor stiffness mapped using shear wave elastography that correlates with an increase of T cell intratumoral migration. Although this treatment alone has minor effects on tumor growth, its combination with anti–PD-1 therapy with X (name of drug) increases the accumulation of effector CD8 T cells and delays tumor progression. This study highlights the rationale of combining approaches targeting the ECM and immune checkpoint proteins.

Keywords: tumor extracellular matrix, anti-PD1, Lysyl Oxidase, T cell migration.

## B032 / Oral cancer vaccine using recombinant Bifidobacterium expressing WT1 protein and anti-PD1 antibody induced WT1 specific immune responses via gut immune system in murine bladder cancer model

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Today, progress of immuno-oncology (I-O) drug has achieved remarkable success for cancer therapy in urological cancers such as renal cancer and urothelial cancers. Immune-checkpoint inhibitors has been considered as one of the most breakthrough in I-O, but induction of tumor-specific immune response is still a fundamental of I-O therapy. Therefore, combining immune-checkpoint inhibitors and cancer vaccines, which can induce tumor-specific CTLs, could be a promising modality to increase response rate in cancer patients. Recently, we developed an oral vaccine platform using recombinant Bifidobacterium longum displaying WT1 protein (B. longum 420) as an oral cancer vaccine [1], and showed anti-tumor effect to bladder cancer in mice in combination with anti-PD-1 antibody. In this study, we investigated the mechanism of the anti-tumor effect of B. longum 420 to demonstrate how the anti-tumor immune responses were induced.

On million cells of MBT-2, a WT1 and PD-L1-postive murine bladder cancer cell line, were subcutaneously inoculated into mice. One week after tumor inoculation, 1×109 of B. longum 420, B. longum 2012 (negative control), or PBS were orally administered 5 times a week for following weeks. Intraperitoneal injection of anti-PD-1 antibody were combined with B. longum 420 administration twice a week. After the treatment, tumors, spleens and mesenteric lymph nodes were collected to analyze the immune respons-

es. As the results, the combination treatment of B. longum 420 and anti-PD-1 antibody induced significantly higher population of tumor infiltrating CD4T cells (0.88%30.12) than B. longum 420 alone (0.46%30.10) ($p<0.05$) and induced substantially higher CD8T cells in MBT-2 tumor. Intracellular cytokine staining showed that B. longum 420 induced remarkably higher population of WT1-CD8-epitope-specific T cell responses in mouse spleens. The combination therapy also induced higher population of IFN-gamma-producing CD4T and CD8T cells in mesenteric lymph nodes after treatments. In addition, oral administration of B. longum 420 increased the expression of CD86 on dendritic cells in mesenteric lymph nodes, suggesting that B. longum induced the maturation of dendritic cells as antigen-presenting cells and initiated T cell immunity in gut immune system to activate the systemic WT1-specific anti-tumor cellular immunity.

In conclusion, we demonstrated that the combination of B. longum 420 oral vaccination and anti-PD-1 antibody could induce the tumor infiltrating T cells and gut immune responses against WT1 in mice. Our findings suggested that our oral cancer vaccine platform could be a promising candidate of WT1 cancer vaccine and combination immunotherapy with immune checkpoint inhibitors including pembrolizumab, an anti-PD-1 antibody therapy for treatment of advanced bladder cancer.

Keywords : Cancer vaccine, Wilms' tumor 1, Immune checkpoint inhibitor, Bladder cancer.

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## B033 / Adjuvant activities of type I interferons

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Immunotherapies such as checkpoint blockade therapy are revolutionizing outcomes for cancer patients. However, further advances are required for the majority of patients to benefit. One reason patients may not respond to checkpoint blockade is due to a suboptimal anti-tumor T cell response. Anti-cancer vaccines are currently being employed to generate robust tumor-specific T cell immunity. A key ingredient for effective vaccination is the inclusion of an appropriate adjuvant that boosts anti-cancer responses, potentially sensitizing patients to checkpoint blockade. We investigated the adjuvant potential of individual members of the type I interferon (IFN) cytokine family using a melanoma whole-cell vaccine approach. To this end, mice received irradiated B16 melanoma cells expressing a single IFN subtype. We have identified that several subtypes demonstrate superior adjuvant potential as compared to the current gold standard adjuvant, polyI :C. In particular, IFNbb induced significantly greater priming and activation of systemic tumor-specific CD8+ T cell responses than polyI :C and all other IFN subtypes tested. This CD8+ T cell expansion is dependent on the presence of CD4+ T cells, CD40/CD40L signalling, and XCR1+ dendritic cells (DCs). Increased tumor-specific T cell infiltration into the tumor microenvironment was observed with IFNb, with infiltrating CD8+ T cells upregulating PD1 expression. Combination of our whole-cell vaccination strategy with anti-PDL1 checkpoint blockade treatment significantly delayed tumor growth. Thus, vaccination strategies incorporating IFNb in combination with anti-PDL1 therapy has the potential to promote strong immune responses and improved therapeutic outcomes for patients.

Keywords : Adjuvant, Type I Interferon, Checkpoint blockers, Melanoma.

## B034 / Mechanism of regulation of double stranded RNA-dependent protein kinase PKR

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Protein Kinase R (PKR) is a double stranded RNA (dsRNA)-dependent kinase that binds to dsRNA leading to its autophosphorylation and concomitant dimerization to the activated state. Upon activation PKR causes translational shutdown by phosphorylating the eukaryotic translation initiation factor eIF2α1. PKR has been shown to be involved in a number of cellular processes such as antiviral immunity and stress response. Due to its role in causing inflammation and apoptosis, it has implications not only in antiviral immunity but also in cancer2. Therefore, regulation of PKR activity is crucial in order to prevent any aberrant activation. Recent reports have shown that A-to-I modification by Adenosine Deaminase Acting on RNA 1 (ADAR1) prevents endogenous RNAs from activating PKR and causing translational shutdown3. Interestingly, a recent study revealed that loss of ADAR1 function in tumors helps in overcoming resistance to immune checkpoint blockade4. It was revealed that loss of ADAR1 activity promoted growth inhibition and tumor inflammation via PKR and MDA5 respectively. In addition to ADAR1, PKR activity is also regulated by a cellular protein called PACT (PKR Activator). However, a complete understanding of how PACT regulates PKR activity is not known.

There are contrasting reports suggesting inhibitory as well as activating effect of PACT on PKR. Our biochemical results with different RNA species show an inhibitory effect of PACT on PKR. PACT comprises of 2 tandem canonical dsRNA-binding domains, that are involved in RNA recognition, and a C-terminal non-canonical dsRNA-binding domain (dRBD3), that is involved in dimerization. Our results show that regulation of PKR activity by PACT is dependent on dRBD3 domain. We have solved the structure of dRBD3 domain of PACT using NMR in collaboration with Chou lab. The structure reveals that PACT dRBD3 forms an asymmetric homodimer with a central 6-stranded beta sheet formed by 3 beta strands from each monomer flanked by the 2 alpha helices on each side. The intramolecular beta sheet has an anti-parallel arrangement whereas the 2 beta strands at the interface are parallelly arranged thereby breaking the dimer symmetry. The structure shows that α2 of monomer B forms a tight network of interactions with β3 and L2 from monomer A. This network of interaction locks monomer B in such a way that the same set of interactions in monomer A are incompatible. These interactions include His285 :Asp297, Gln271 :Gln304 and Glu263 :Gln304. Therefore, we propose a model of dimeric asymmetry for PACT-D3 wherein the interaction at the A :B interface prevents the analogous interaction at the B :A interface leading to two modes of dimers. Interestingly, the NMR data revealed that there is a constant exchange between the two dimeric states. We are currently in the process of doing some mutational and functional studies to understand the role of the PACT dimerization domain in regulation of PKR activity.

Keywords : Innate immunity, PKR.

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## B035 / Modulating the tumor microenvironment to improve immunotherapy : Interrogating the interplay between MYC and immune checkpoints in pancreatic cancer

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Immunotherapy with antibodies that target the PD-1/PD-L1 axis has shown great promise and even curative outcomes in many cancer subtypes but not in pancreatic ductal adenocarcinoma (PDAC). This is due in large part to the lack of functional immune cells in the characteristically dense stroma in PDAC, resulting in an immune-quiescent ("cold") tumor microenvironment (TME). Amplified expression of MYC in tumors has been shown to directly upregulate PD-L1 and other immune checkpoints and orchestrate immunosuppression of the TME. Although MYC has been suggested as a global regulator of the immune response, it is not known how MYC-targeted therapies specifically affect the immune cells within the TME, or if these types of therapies could be leveraged to improve immunotherapeutic outcomes in PDAC. Given that

PDAC is one of the deadliest and fastest-moving cancers — with a 5-year survival rate of less than 10% — there is a clinically unmet need to understand the mechanism by which targeted therapies affect the immune microenvironment. We aim to solve this problem by treating syngeneic PDAC with MYC-targeted therapies and studying the mechanism by which they affect different cell populations in the TME. Since oncogenic MYC is highly expressed in "cold" PDAC tumors, our central hypothesis is that MYC-targeted therapy will recruit T cells to the tumor, making the tumors "hot, " and improving the therapeutic effects of anti-PD-L1 immunotherapy. A panel of murine and human PDAC cells were drugged with MYC-targeted therapies (BET inhibitor JQ1, ERK inhibitor SCH772984, and MEK inhibitor trametinib) to observe the effects on PD-L1 and transferrin receptor (TfR), a surrogate marker for MYC expression. We found a significant increase of PD-L1 expression ($P < 0.001$) in KRAS-mutant PDAC cells that respond to ERK inhibitor SCH772984 and MEK inhibitor trametinib (measured by a significant decrease of TfR; $P < 0.05$). In these same cells, we found a significant decrease in PD-L1 expression upon response with BET inhibitor JQ1 ($P < 0.001$). Interestingly, we observe the opposite effect in KRAS wild-type PDAC cells, with essentially no significant change in PD-L1 expression with a response to ERK and MEK inhibition (as measured via decrease in TfR expression), coupled to significant increase of PD-L1 ($P < 0.001$) upon treatment with JQ1. To examine the effects on the TME as a whole, we created a molecular imaging agent from an anti-PD-L1 antibody (clone 6E11, Genentech) to noninvasively and longitudinally assess tumor burden in vivo. Anti-PD-L1 was conjugated to desferrioxamine (DFO) and radiolabeled with zirconium-89 (89Zr) for positron emission tomography (PET) imaging. KPC cells (established from genetically engineered mouse models p48-Cre;KRASLSL-G12D; p53R172H/+) were infected with luciferase-GFP lentivector and orthotopically implanted into the pancreas of C57BL/6J mice. Tumor-bearing KPC-Luc-GFP mice were treated with ERK inhibitor SCH772984 (90 mg/kg once daily for 6 days) and injected with [89Zr]Zr-anti-PD-L1 to undergo serial PET imaging. We observed significantly increased uptake of [89Zr]Zr-anti-PD-L1 ($P < 0.05$) upon drug treatment compared to vehicle control at 72 h post-injection of radiotracer. These findings were correlated with increased expression of PD-L1 by ex vivo immunofluorescence, along with an increase in PD-L1+ and CD3+ T cells in the TME, as measured by ex vivo flow cytometry ($P < 0.05$). Further mechanistic investigations are being pursued to identify the functionality of T cell infiltration (by probing CD4, CD8, and other T cell fates) both in vitro in activated T cells isolated from splenocytes as well as ex vivo (through additional probing of KPC-Luc-GFP mice treated with MYC-targeted therapy). We anticipate that our mechanistic studies along with guidance through [89Zr]Zr-anti-PD-L1 PET will be able to drive synergistic combinatorial therapeutic approaches with immune checkpoint blockade in PDAC.

Keywords: MYC, pancreatic cancer, tumor microenvironment, immune checkpoint.

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## B036 / Tumor Treating Fields (TTFields) induce immunogenic cell death resulting in enhanced antitumor efficacy when combined with anti-PD-1 therapy

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Tumor Treating Fields (TTFields) are a clinically applied anti-neoplastic treatment modality delivered via noninvasive application of low-intensity, intermediate-frequency, alternating electric fields. In this study we evaluated whether TTFields-induced cell death can be immunogenic and therefore suitable for combination with anti-PD-1 therapy. Cancer cells were treated with TTFields using the inovitro system. Immunogenic cell death (ICD) was characterized by the exposure of calreticulin on the cell surface, secretion of ATP, and release of HMGB1. For detection of ER stress, phosphorylation of eIF2α was assessed. TTFields effect on autophagy was evaluated using electron microscopy, and evaluation of LC3. Bone marrow derived dendritic cells (DCs) were co-incubated with TTFields treated cells and phagocytosis by DCs and DCs maturation were evaluated. The combination of TTFields and anti-PD-1 was evaluated in short duration treatment protocol in orthotopic lung cancer model and long duration treatment protocol in subcutaneous colon cancer model. Analysis of infiltrating cells was performed using flow cytometry.

We demonstrate that cancer cells that die during TTFields application exhibit ER stress leading to calreticulin translocation to the cell surface, as well as release of damage-associated molecular patterns including HMGB1 and ATP. Moreover, we show that TTFields treated cells promote phagocytosis by DCs, DCs maturation in vitro, and promote immune cells recruitment in vivo. We also show that the combined treatment of TTFields plus anti-PD-1 led to a significant decrease in tumor volume and significant increases in CD45+ tumor infiltrating cells in both tumor models. In the lung tumors, these infiltrating cells, specifically macrophages and DCs, demonstrated upregulation of surface PD-L1 expression following short treatment duration. Correspondingly, cytotoxic T-cells isolated from these tumors have shown higher levels of IFN-γ production relative to untreated mice. In the colon cancer tumors, significant increases in T-cell infiltration was observed following long treatment duration with TTFields plus anti-PD-1.

Our results demonstrate the potential of TTFields therapy to induce ICD. We also demonstrate robust efficacy of concurrent application of TTFields and anti PD-1 therapy in mouse models of cancer. These data suggest that combining TTFields with anti-PD-1 might achieve tumor control by further enhancing antitumor immunity.

Keywords: Tumor Treating Fields, Anti-PD-1 Therapy, Immunogenic Cell Death.

## B037 / Combining anti-Angiopoietin-2 and anti-PD-1 therapy with VEGF/VEGFR signaling blockade prolongs overall survival in small cell lung carcinomas

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Lung cancer is the leading cause of smoking- and cancer-related deaths worldwide. Small cell lung cancer (SCLC) accounts for approximately 15 % of all newly diagnosed lung cancers, is driven by bi-allelic loss in RB1 and TP53 and represents the most aggressive pulmonary and neuroendocrine differentiated carcinoma of the lung. Current chemotherapies are initially effective against small cell lung carcinomas however, tumors rapidly reoccur and patients die within only a few months. Recently, programmed death ligand 1 (PD-L1) positive late stage SCLC patients received a high-affinity monoclonal programmed death receptor 1 (PD-1) antibody reactivating T cells responses against the tumor cells. Despite remarkable response rates, most SCLC patients harbor a primary resistance or acquire resistance during treatment by resistance mechanisms triggered by the tumor cells themselves or by an immune suppressive tumor microenvironment. Thus, there is a critical need to combine immune checkpoint blockade with other therapies to overcome these resistances.

We implemented a combined therapy concept including anti-Angiopoietin-2 and anti-PD-1 monoclonal antibodies and VEGF/VEGFR signaling blockade for SCLC in an autochthonous mouse

model. Thereby, Angiopoietin-2 is known as a negative prognostic marker in SCLC and may serve as biomarker for immune checkpoint targeted therapy. As a read out, we used X-ray computed tomography, flow cytometry and end point immunohistochemistry.

We found that this triple combination treatment significantly improved survival of mice suffering from SCLC, compared to corresponding monotherapies. Moreover, we found enhanced T cell infiltration into the tumors and an acquired resistance mechanism to anti-PD-1 antibody monotherapy. In line with the results obtained from our mouse model, we found similar results in peripheral blood mononuclear cells of SCLC patients with acquired adaptive resistance to PD-1 blockade by Nivolumab.

Taken together, there is evidence for a substantial benefit of implementing combined blockade of immune checkpoints and tumor-angiogenesis in the treatment of patients suffering from SCLC in order to overcome acquired resistance to immune checkpoint blockade.

Keywords: Small cell lung cancer, PD-1, VEGFR signaling, Angiopoietin-2.

## B038 / Smac mimetics synergize with anti-CTLA-4 to eradicate bladder cancer

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The Inhibitors of Apoptosis (IAPs) are oncogenes that enable cancer cells to evade numerous death signals, including signals originating from the immune system. Smac mimetic compounds are IAP antagonists that cause the degradation of two IAPs, cIAP1 and cIAP2, which are proteins important to immunity and cancer cell survival. To kill cancer cells, Smac mimetics require an immunological death ligand, such as Tumor necrosis factor alpha (TNFα). In addition, Smac mimetics modulate inflammation and immunity by regulating the activity of the alternative Nuclear Factor Kappa B (NF-κB) signaling pathway, which is critical to the control of immune cell activity. We are exploring the combination of Smac mimetics with immune checkpoint inhibitors for their effects in various animal tumor models, including invasive bladder cancer. Our data shows that the combination of Smac mimetics with an immune checkpoint inhibitor specific for cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is effective at generating long-term durable cures in all dually-treated mice bearing MB49 bladder cancer. We demonstrate that anti-CTLA4 from immunoglobulin subtypes with antibody dependent cytotoxicity (ADCC) activity show improved anticancer responses in tumor bearing mice, when compared to those anti-CTLA4 monoclonal antibodies that lack ADCC. We also observed that the combination of Smac mimetics with ADCC-active anti-CTLA4 have reduced levels of immunosuppressive regulatory T cell (Tregs) within the tumor microenvironment. The combination therapy enhanced a compensatory shift in the exhaustion profile of intratumoral T cells to a more reinvigorated phenotype compared to either monotherapy. Taken together, Smac mimetics exploit the lack of Treg-mediated suppression on key immune effectors in the tumor microenvironment following anti-CTLA-4 therapy to effect their action. Our findings from these pre-clinical studies will guide the use of Smac mimetic and immune checkpoint inhibitor based immunotherapies for the treatment of invasive bladder cancer.

Keywords: Inhibitors of apoptosis (IAPs), Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), Bladder cancer.

## B039 / Interim results from CLASSICAL-Lung, a phase 1b/2 study of pepinemab (VX15/2503) in combination with avelumab in advanced NSCLC patients who progressed on prior anti-PDx therapy

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Despite progress of immune checkpoint blockade therapies, many non-small cell lung cancer (NSCLC) patients do not receive durable clinical benefit from these agents, and even in those who do respond initially, acquired resistance and tumor recurrence can develop. Therefore, the development of therapies that can overcome resistance factors remains a critical unmet need. Pepinemab is an IgG4 humanized monoclonal antibody targeting semaphorin 4D (SEMA4D, CD100). In vivo preclinical models demonstrated antibody blockade of SEMA4D promoted immune infiltration and reduced function and recruitment of immunosuppressive myeloid cells within the tumor. Importantly, preclinical combinations of anti-SEMA4D with various immunotherapies enhanced T cell infiltration and activity, as well as durable tumor regression. The CLASSICAL-Lung clinical trial evaluates the combination of pepinemab with PD-L1 antibody avelumab to couple beneficial modifications of the immune microenvironment via pepinemab with immune activation via checkpoint inhibition. This ongoing phase 1b/2, open label, single arm, first-in-human combination study is designed to evaluate the safety, tolerability and efficacy of pepinemab in combination with avelumab in patients with advanced (stage IIIB/IV) NSCLC (NCT03268057), including a dose escalation cohort and expansion cohorts consisting of 1) 17 immunotherapy-naïve patients and 2) the focus of the present abstract, 33 patients whose tumors progressed during or following immunotherapy (IO failure). Patients in expansion cohorts received the recommended phase 2 dose of pepinemab (10 mg/kg, q2W) in combination with avelumab (10mg/kg, Q2W) by intravenous infusion. The combination was well tolerated with no concerning safety signals identified to date. No patients experienced a treatment related adverse event (TRAE) leading to study discontinuation or death and the most frequent related AEs were grades 1 or 2 fatigue, pyrexia, or chills. One dose limiting toxicity occurred, a grade 3 pulmonary embolism in the 10mg/kg pepinemab cohort, and resolved without recurrence. Interim analysis focusing on the greatest unmet need includes 22 evaluable patients within the IO failure cohort. Two patients experienced a partial response (PR) of 49% and 37% tumor reduction on study following acquired resistance to prior treatment with pembrolizumab. In addition, stable disease of at least 8 weeks was observed in 11 patients and 4 patients have remained on study for ≥20 weeks. Analysis of pre- and on-treatment lung biopsies demonstrated no or low tumor burden detected in 2 biopsies from patients with PR, and no detectable tumor was observed in the biopsies from 3 of 4 samples assessed from patients with stable disease. Furthermore, an increase in CD8+ T cell density and CD8:FoxP3 ratio was observed following treatment with pepinemab + avelumab. Preliminary data suggest the combination of pepinemab plus avelumab is well tolerated and shows initial signals of antitumor activity in patients who did not respond to or developed resistance to prior anti-PDx therapy. Ongoing analysis to be presented includes updated clinical response data, as well as additional immunophenotyping of tissue biopsies, including but not limited to activated T cells, regulatory T cells, DCs, monocytes, macrophages, and importantly myeloid-derived suppressor cells (MDSCs).

Keywords: Semaphorin, immune checkpoint, immunotherapy resistance, MDSC.

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1. Evans EE et al. 2015. Antibody blockade of semaphorin 4D promotes immune infiltration into tumor and enhances response to other immunomodulatory therapies. Cancer Immunol Res. 3: 689-701 2. Clavijo PE et al. 2019. Semaphorin4D inhibition improves response to immune checkpoint blockade via attenuation of MDSC recruitment and function. Cancer Immunol Res. 7(2):282-291.

## B040 / A novel antibody targeting ICOS increases intratumoural cytotoxic to regulatory T cell ratio and induces tumour regression

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The immunosuppressive tumour microenvironment constitutes a significant hurdle to the response of immune checkpoint blockers. Both soluble factors and specialised immune cells such as regulatory T cells (TReg) are key components of intratumoral immunosuppression. Previous studies have shown that Inducible Co-Stimulatory receptor (ICOS) is highly expressed in the tumour microenvironment, especially on TReg, suggesting that

it represents a relevant target for preferential depletion of these immunosuppressive cells. Here, we used immune profiling of samples from tumour bearing mice and cancer patients to first fully characterise the expression of ICOS in different tissues and solid tumours. By immunizing an Icos knockout transgenic mouse line, we selected a fully human IgG1 antibody called KY1044 that binds ICOS from different species. Using KY1044, we demonstrated that we can exploit the differential expression of ICOS on T cell subtypes to modify the tumour microenvironment and therefore improve the anti-tumour response. We showed that KY1044 induces sustained depletion of ICOShigh TReg cells in mouse tumours and depletion of ICOShigh T cells in the blood of non-human primates, but also stimulates the secretion of pro-inflammatory cytokines from ICOSlow TEFF cells. Altogether, KY1044 improved the intratumoral TEFF:TReg ratio and increased activation of TEFF cells, resulting in monotherapy efficacy or in synergistic combinatorial efficacy when administrated with the immune checkpoint blocker anti-PD-L1. In summary, our data demonstrate that targeting ICOS with KY1044 can favourably alter the intratumoral immune contexture, promoting an anti-tumour response.

Keywords: ICOS, Tregs, PD-L1.

## B041 / AVID200 neutralizes TGF-beta1 and -beta3, the principal immunosuppressive TGF-beta isoforms overexpressed by tumors and sensitizes tumors to immune checkpoint inhibitors

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AVID200 is a novel, rationally-designed receptor ectodomain trap that inhibits transforming growth factor-beta (TGF-beta) isoforms 1 and 3 with pM potency. Selective targeting of isoforms 1 & 3 with AVID200 has the potential to increase the number of patients that benefit from immune checkpoint inhibitors (ICIs). ICIs, such as anti-PD-(L)1 therapies, are approved in a multitude of oncology indications, including as a first line treatment for advanced stage disease. However, only a fraction of patients have durable responses, rendering primary and acquired resistance to ICIs a major unmet medical need. The TGF-beta pathway has been directly associated with resistance to ICIs. TGF-beta acts by inhibiting the recruitment and activation of anti-tumor T-cells, either directly, or indirectly through its action on cancer-associated fibroblasts which promotes fibrosis in the tumor microenvironment. Hence, targeting of TGF-beta represents a novel strategy to increase sensitivity of tumors to treatment with ICIs. In this study, the percent of patients exhibiting tumor over-expression (>30FPKM) of TGF-beta isoforms was analyzed in >10, 000 samples from the TCGA RNAseq database. The potency and selectivity of AVID200 and, for comParison, other clinical stage TGF-beta inhibitors, were assessed in vitro using an A549 cell-based assay. The ability of AVID200 to enhance the tumor-cell killing activity of T-cells was evaluated in vivo in a syngeneic 4T1 cancer model. In addition, the capacity of AVID200 to enhance the efficacy of ICIs was assessed in EMT-6 and MC-38 cancer models. TGF-beta1 and -beta3 were found to be the predominant isoforms expressed in solid tumors, with TGF-beta2 showing only minimal expression. Hence, for maximal efficacy, both TGF-beta1 and -beta3 isoforms should be targeted, particularly since they can functionally substitute for each other. In vitro studies showed that AVID200 exhibited superior potency (low pM) and selectivity for TGF-beta1 & -beta3 vs TGF-beta2 as compared to the other clinical stage TGF-beta inhibitors tested. AVID200 increased the response to anti-PD-L1 in vivo and enhanced T-cell mediated killing of tumor cells in a dose dependent manner. In agreement with these findings, AVID200 was also shown to promote T-cell infiltration in vivo.

In conclusion, AVID200 efficiently neutralizes TGF-beta1 and -beta3, the main oncogenic isoforms of TGF-beta, with best-in-class potency and sensitizes tumors to immune checkpoint blockade. AVID200 has minimal activity on TGF-beta2 which is desirable since TGF-beta2 inhibits metastasis by promoting tumor cell dormancy, is required for normal cardiac homeostasis, and is a positive regulator of hematopoiesis. A Phase 1 dose-escalation study with AVID200 in patients with solid tumors is ongoing.

Keywords: tumor microenvironment, TGF-beta.

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## B042 / Long-acting MDNA109: Emerging IL-2 Superkines displaying potent anti-tumoral responses.

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Background: Use of cytokines as cancer therapy was initiated with the approval of interleukin-2 (Proleukin®) for treatment of metastatic melanoma and renal cell carcinoma. However, the therapeutic utility of Proleukin® remains limited due to a short half-life and toxicity. In addition, Proleukin® preferentially induces the unwanted activation of regulatory T cells (Tregs), which express the high-affinity trimeric IL-2 receptor (CD25/CD122/CD132). In contrast, NK and naïve CD8 T cells express the intermediate-affinity form (CD122/CD132) of the receptor, towards which IL-2 has reduced potency. To overcome these limitations, we engineered long-acting variants of the IL-2 Superkine MDNA109 (MDNA109-LA, MDNA109-LA1 and MDNA109-LA2) fused to an inactive protein scaffold, capable of selectively activating cytotoxic T cells with enhanced potency while displaying diminished effects on Tregs.

Methods: To test the activities of MDNA109-LA, MDNA109-LA1 and MDNA109-LA2, a series of in vitro and in vivo studies were conducted, including IL-2 pathway signaling in human PBMCs, Biacore binding analyses and tumor growth inhibition studies in tumor models with or without immune checkpoint inhibitors (ICIs).

Results: The biochemical activities of long-acting MDNA109 Superkines were assessed via STAT5 phosphorylation using human PBMCs derived from healthy donors. MDNA109-LA selectively triggered enhanced STAT5 signaling in naïve CD8 T cells compared to native IL-2. In a pre-established CT26 colon cancer model, MDNA109-LA displayed strong tumor growth inhibition, and combination treatments with ICIs, particularly anti-CTLA-4, resulted in a majority of mice being tumor free. To further improve the potency of MDNA109-LA, a second generation molecule, MDNA109-LA1, was engineered to mitigate binding to CD25. Biacore studies confirmed lack of binding of MDNA109-LA1 to CD25. In PBMC studies, MDNA109-LA1 greatly reduced activation of Tregs while maintaining its potency to activate naïve CD8 T cells. In CT26 and B16F10 (mouse melanoma) models, treatment with MDNA109-LA (bi-weekly for 2 weeks) and MDNA109-LA1 (once weekly for 2 weeks) potently inhibited tumor growth. These data suggest that MDN109-LA1 could lead to potent therapeutic effects with a dosing schedule similar to that used for ICIs. MDNA109-LA2 contains the same backbone as MDNA109-LA but is fused to an alternative protein scaffold to further improve PK and retention in the tumor microenvironment. MDNA109-LA2 inhibited growth of B16F10 tumors to a similar extent as MDNA109-LA and MDNA109-LA1. When administered on the same dosing regimen as MDNA109-LA, MDNA109-LA2 displayed superior tumor growth inhibition in the CT26 model. The combination of MDNA109-LA2 and anti-CTLA-4 resulted in complete tumor regression in 8 of 9 mice (89%). These mice remained tumor free and were highly resistant to two additional CT26 re-challenges with-

out further treatment, consistent with development of long-term memory response. Analyses of tumor-infiltrating lymphocytes, cytokine profiles in immune cells as well as PK/PD studies are currently underway. Additional data to support lead candidate selection for IND enabling studies and first in human clinical trials will also be presented within the context of other approaches to improve PK and PD properties of IL-2.

Conclusion: MDNA109 is the only IL-2 in development that has been engineered for high affinity binding to CD122. Furthermore, three long-acting Superkines, MDNA109-LA, MDNA109-LA1, and MDNA109-LA2 have superior potency over IL-2 at activating NK and naïve CD8 effector T-cells. These 3 molecules potently inhibited CT26 and B16F10 tumor growth in vivo as monotherapies as well as in combination with ICIs on a dosing schedule that is similar to that of antibody based therapies. MDNA109-LA1 in particular has greatly reduced activity towards Tregs, which could potentially translate to superior anti-tumor activity and a better safety profile.

Keywords: MDNA109, IL-2, Superkine, immune checkpoint inhibitors.

## B043 / Dramatically improving vaccinia virus-based cancer immunotherapeutics by deleting E5R, which encodes a dominant viral inhibitor of cGAS

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Viral-based cancer immunotherapies have gained momentum in the era of immune checkpoint blockade (ICB). Both preclinical and clinical studies have shown that oncolytic viral therapy is an effective way to overcome resistance to ICB by turning “cold” tumors that are non-responsive to ICB to “hot” tumors that are responsive to ICB. Both non-replicative and replicative oncolytic virus can induce antitumor immunity through multiple mechanism of actions including: (i) the induction of immunogenic cell death; (ii) the release of tumor-associated antigens (TAAs) from dying tumor cells as well as damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs); (iii) the alteration of immunosuppressive tumor microenvironment by activating tumor-infiltrating dendritic cells (DCs) and T cells, and (iv) the depletion of immune suppressive cell populations. We have previously shown that the cGAS/STING-mediated cytosolic DNA-sensing pathway and Batf3-dependent cross-presenting DCs play important roles in the generation of antitumor immunity by intratumorally delivered inactivated modified vaccinia virus Ankara (iMVA) (Dai et al., Science Immunology, 2017). MVA is a highly attenuated vaccinia strain that is replication incompetent in most mammalian cells. Wild type vaccinia (WT VACV) is highly immune suppressive to infected DCs due to the expression of many immune evasions proteins. To improve the immunogenicity of MVA or vaccinia, we screened 72 vaccinia viral early genes for their abilities to inhibit the cGAS/STING pathway in an IFNB promoter-based luciferase assay. We identified vaccinia E5 (encoded by the E5R gene) is a dominant inhibitor of cGAS. Infection of bone marrow-derived DCs (BMDCs) with MVA deleting the E5R gene (MVAΔE5R) induced much higher levels of IFNB gene expression and protein secretion compared with either MVA or inactivated MVA. MVAΔE5R strongly activates DCs and induces DC maturation as well as their abilities to prime and activate antigen-specific T cells in a cGAS-dependent manner. In addition, MVAΔE5R infection of DCs causes much less cell death compared with those infected with either MVA or inactivated MVA. Recombinant VACV deleting the E5R gene (VACVΔE5R) is 1000-fold more attenuated compared with WT VACV in an intranasal infection model. Although WT VACV does not induce IFN production in BMDCs, infection with VACVΔE5R induces higher levels of IFNB gene expression and protein secretion compared with MVA.

We have now generated two platforms of vaccinia-based cancer immunotherapeutics- one is recombinant MVA (rMVA) with deletion of the E5R gene expressing two transgenes: a DC growth factor Flt3L and T cell co-stimulatory molecule OX40L; the other one is recombinant VACV (rVACV) with deletion of the thymidine kinase and E5R genes expressing anti-CTLA-4 antibody, Flt3L, and OX40L. Both rMVA and rVACV infection of B16-F10 tumor cells induces ATP release, which is a marker for immunogenic cell death. Intratumoral injection of either rMVA or rVACV generates strong systemic antitumor effects in multiple murine syngeneic tumors models either alone or in combination with systemic ICB therapy. Taken together, these results indicate that deleting the E5R gene from MVA or VACV genome dramatically improved the immunogenicity of the recombinant viruses.

Keywords: immunogenic DNA virus, Modified vaccinia virus Ankara, in situ vaccination, cGAS/STING.

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Dai, P., W. Wang, N. Yang, C. Serna-Tamayo, J.M. Ricca, D. Zamarin, S. Shuman, T. Merghoub, J.D. Wolchok, and L. Deng. (2017). Intratumoral delivery of inactivated modified vaccinia virus Ankara (iMVA) induces systemic antitumor immunity via STING and Batf3-dependent DCs. Science Immunology 2, eaal1713: 1-15.

## B044 / Cyclophosphamide chemotherapy enhances the anti-tumor potency of GITR engagement

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Enhancing anti-tumor immunity with immune checkpoint blockade has become a standard apporach for some malignancies, but primary and secondary resistance to CTLA-4 and PD-1 blockade limit the number of patients that show clinical response. Exploring novel pathways that orchestrate an anti-tumor immune response could increase the clinical impact of immunotherapy strategies. Glucocorticoid-induced TNFR family related gene (GITR) is a co-stimulatory molecule expressed primarily on the surface of T cell subsets. GITR engagement promotes potent activation, proliferation, and clonal expansion of effector T cells while hampering the suppressive function of regulatory T cells. However, clinical trials with agonist anti-GITR antibodies given as a monotherapy have yielded disappointing results. We hypothesized that immunomodulatory chemotherapy regimens could enhance the anti-tumor efficacy of GITR antibodies. Cyclophosphamide (CTX) is an alkylating agent known to deplete T cell subsets while causing homeostatic proliferation of antigen-specific effector T cells. CTX also modulates GITR expression on T cell subsets in a dose dependent manner. GITR engagement with the monoclonal antibody DTA-1, in combination with a single dose of CTX, promotes potent anti-tumor immunity in multiple transplantable murine tumor models including B16 melanoma, MCP-11 myeloma, and CT26 colon carcinoma. Specifically, CTX triggers tumor necrosis, releasing antigen that can prime anti-tumor immunity. Additionally, combination therapy increases the ratio of CD8+ effector T cells to Tregs, at least in part by inducing Treg-specific activation-induced cell death. Single cell sequencing and flow cytometry demonstrate a marked increase in pro-inflammatory cytokines, cytolytic granules, and activation markers accompanied by a reduction in exhaustion markers in intratumoral CD8+ T cells. Overall, we demonstrate that a single dose of cyclophosphamide added to GITR engagement is a potent combinatorial approach that primes an anti-tumor response, enhances T cell fitness, and shifts the balance between effector and suppressive T cells to control tumor growth, an approach that is now incorporated into an ongoing clinical trial (NCT03861403).

Keywords: GITR, Cyclophosphamide.

## B045 / Immunomodulatory effects of metronomic oral oxaliplatin using nanoemulsion and its combination with aPD-1 therapy

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A new type of orally available oxaliplatin nano-emulsion (NE) was developed by formulating a complex of negatively charged oxaliplatin with positively charged DCK (deoxycholic acid-lysine conjugates), which is a derivative of deoxycholic acid. This formulated oxaliplatin showed significantly higher oral bioavailability than without formulation. Oral anticancer drugs considered for metronomic chemotherapy have known to show immunological anti-cancer effects. In particular, these drugs can induce not only apoptosis but also immunogenicity in tumor. This means that oral oxaliplatin-NE could have immunological synergistic effects with 𝛼PD-1, an immune checkpoint inhibitor. Then B16F10.OVA murine melanoma cell line was selected to evaluate how oxaliplatin-NE induced immunogenicity in vitro. As a result of treating oxaliplatin-NE, calreticulin was exposed to cell membrane and HMGB-1 was translocated to extracellularly, a marker of immunological cell death. When oral oxaliplatin-NE was metronomically treated to xenograft model in vivo, the drug-treated group showed higher amount of expressed HMGB-1 than that of control group. This means the oral metronomic oxaliplatin-NE was proven to have immunogenicity. Based on these results, antitumor synergistic effect of oral oxaliplatin-NE with 𝛼PD-1 was evaluated. The 𝛼PD-1 combination group showed significantly higher antitumor effect than each individually treated group. Also, oral metronomic treated mice showed better antitumor effect than conventional intravenous injection treated mice. In the aspect of immunological effects, the oxaliplatin-NE helped to activate innate immune response, showing activation of dendritic cells and increased antigen uptake in the tumor draining lymph node. This even could affect the adaptive immunity, showing increased number of CD45, CD8 T cells in tumor. The drug also improved the function of T cells as a result of increased ratio of PD-1-/INFr+ CD8 T cells.

In conclusion, orally metronomic oxalipaltin-NE stimulated stronger immunogenicity in both local tumor and systemically, then it encouraged further anticancer effects. Furthermore, oxaliplatin-NE showed significant immunologic synergistic effects when used with immunotherapy, such as 𝛼PD-1 antibody.

Keywords: oral metronomic therapy, oxaliplatin, nanoemulsion.

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## B046 / Uncovering response prediction to checkpoint inhibitor based immune therapies by non-invasive in vivo 64Cu-NODAGA-CD69-mAb positron emission tomography (PET)/magnetic resonance imaging (MRI)

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T cell infiltration and activation is essential for successful cancer immunotherapies and determines antitumoral response to immune checkpoint inhibitor treatment (CIT). Beside surgical resection, radiation therapy and chemotherapy, CIT has become a standard of care treatment approach to date for various cancers such as metastatic malignant melanoma. Nevertheless, despite the enormous success of CIT the majority of cancer patients do not benefit at all and response to CIT still remains poorly predictable. As T cells express the early activation antigen CD69 upon stimulation, CD69 might be an excellent candidate for monitoring immune responses by non-invasive in vivo molecular imaging(Cibrian et. al, 2017). The aim of this study was to develop a clinically translatable antibody based CD69 targeting immunoPET tracer and to evaluate its potential as a novel imaging tool for monitoring T cell activation by CIT.

We used the radiochelator NODAGA for copper-64 (64Cu) radiolabeling of the anti CD69-monoclonal antibody (mAb). Immunoreactivity assays were performed on in vitro activated and non-activated mouse CD4+ and CD8+ T cells. C57BL/6N mice with subcutaneous MC38 colon adenocarcinomas (responsive to CIT) or OVA-B16 melanomas (non-responsive to CIT) with a tumor diameter of 5-8 mm underwent a combined CIT by weekly intraperitoneal (i.p.) injection of anti-programmed death ligand 1 (anti-PD-L1) mAbs and anti-lymphocyte activation gene 3 (anti-LAG-3) mAbs. Another group of OVA-B16 tumor bearing mice underwent a combined immunotherapy consisting of cyclophosphamide + adoptively transferred IFN-g-producing OVA-specific CD4+ (Th1) T cells + anti-PD-L1 mAbs + anti-LAG-3 mAbs. Control groups of both tumor models received no therapy. On three different timepoints after therapy start simultaneous PET/MRI was acquired 48 hours after i.v. 64Cu-NODAGA-CD69-mAb injection.

Radiochelation of the 64Cu-NODAGA-CD69-mAb yielded high radiolabeling efficiency (> 95%) and immunoreactivity (> 60%). Non-invasive in vivo PET/MRI investigations of CIT-treated MC38 tumor bearing mice revealed a significant increase in 64Cu-NODAGA-CD69-mAb tumor uptake on day 3 (13.6 %ID/cc 3 1.1 SEM) and 5 (13.4 %ID/cc 3 0.6) after onset of CIT when compared to the control group with a similar tumor volume (10.3 %ID/cc 3 0.3 SEM). In contrast, no differences between the CIT- (10.5 %ID/cc 3 0.2 SEM) and the control group were observed on day 8, highlighting the temporal dynamics of CD69 as an early activation marker. Moreover, we measured no significant difference of the 64Cu- NODAGA-CD69-mAb uptake in spleen, liver and kidney uptake between the experimental groups. OVA-B16 tumor bearing mice which did not respond to CIT treatment exhibited no increase in tumor 64Cu-NODAGA-CD69-mAb uptake on day 3 when compared to the control group (11.6 %ID/cc 3 0.3 SEM vs. 10. 9 %ID/cc 3 0.4 SEM). In contrast, efficiently with cyclophosphamide + OVA-Th1 cells + PD-L1 mAbs + LAG-3 mAbs treated OVA-B16 tumor bearing mice exhibited a significantly increased 64Cu-NODAGA-CD69-mAb tumor uptake (13.81 %ID/cc 3 0.5 SEM) when compared to the control (10.9 %ID/cc 3 0.4 SEM) or combined CIT-treatment (11.6 % ID/cc 3 0.3 SEM) group. Ex vivo biodistribution analysis confirmed our in vivo PET results.

Whole body 64Cu-NODAGA-CD69-mAb-PET/MRI enables monitoring of T cell activation and thus identifying CIT responders as early as 3 days after onset of CIT. Consequently, immediate identification of CIT resistance by 64Cu-NODAGA-CD69-mAb-PET/MRI facilitates early treatment modification and will probably guide clinicians to improve the survival of cancer patients.

Keywords: Programmed cell death ligand 1, Lymphocyte activation antigen 3, PET/MR, T-cells.

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## B047 / PGE2/COX2 pathway inhibition enhances anti-melanoma immunity and synergizes with anti-PD1 immunotherapy

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Checkpoint inhibitors such as anti-PD1 (aPD1) therapies have revolutionized treatment of metastatic melanoma, but a large subset of patients receiving such treatments fail to respond to aPD1

monotherapy. The PGE2/COX2 signaling pathway is one of the many pathways implicated in T cell exhaustion and PD1/PD-L1 up-regulation and thus represents an attractive pharmacologic target to enhance effects of aPD1 therapy due to the availability and safety of inhibitors such as aspirin or NSAIDs. There is evidence that PGE2/COX2 pathway inhibitors act synergistically with aPD1 therapy in murine melanoma and breast cancer models to bolster response rates. Here we further attempted to further characterize the synergism between PGE2/COX2 pathway inhibitors and aPD1 therapy and elucidate its mechanism using the YUMMER (Yale University Mouse Melanoma Exposed to Radiation) 1.7 model, an irradiated, syngeneic cell line originating from BrafV600E; Pten-/-; and Cdkn2a-/- genetically engineered mouse melanomas. YUMMER1.7 cells implanted into the flanks of C57BL6/j mice show reproducible but partial responses to intraperitoneal aPD1 therapy due to their irradiation-induced neoantigen burden, while YUMM1.7 cells, which have not been irradiated, do not respond to aPD1 therapy and model immunologically "cold" tumor cells. The YUMM/YUMMER model thus serves as an ideal platform to study if and how concurrent PGE2/COX2 pathway blockade may result in additive effects to aPD1 therapy. 6-8 week old male C57BL6/j mice were injected with 500K YUMMER1.7 or YUMM1.7 cells and treated with intraperitoneal aPD1 therapy alone starting on day 7 after tumor implantation (n = 10), ibuprofen dissolved in drinking water at a concentration of 1 mg/mL started on the day of tumor implantation (n = 10), combination aPD1/ibuprofen therapy (n = 10), or untreated (n = 10). Tumor growth was monitored biweekly and tracked to an endpoint of 1cm3. In a separate cohort, IFN-gamma depletions were performed biweekly for aPD1 alone, ibuprofen alone, and aPD1/ibuprofen combination groups at a dose of 0.2 mg/mouse starting on the day of tumor implantation. Plasma and tumor samples for cytokine analysis were harvested at day 12 post tumor implantation after 2 doses of aPD1. Survival was significantly increased ($p < 0.0001$) in mice implanted with YUMMER1.7 receiving either ibuprofen alone (80%) or combination aPD1/ibuprofen (100%) after 60 days compared to mice receiving aPD1 alone (0%). 50% of aPD1-nonresponsive YUMMER1.7 tumors regressed when ibuprofen was added at the designated outgrowth threshold of 150mm3. In mice implanted with YUMM1.7, the combination of aPD1 and ibuprofen significantly extended median survival to 34 days as compared to 24 days with aPD1 alone or ibuprofen alone ($p = 0.0002$). Gamma blockade in YUMMER1.7 nullified the antitumor response of ibuprofen alone, aPD1 alone, and the aPD1/ibuprofen combination, suggesting that ibuprofen exerts its antitumor effects via a gamma-dependent mechanism. Furthermore, combining ibuprofen with aPD1 therapy resulted in significant increases in the gamma-dependent cytokine CXCL10 ($p = 0.018$) and IFN-B1 ($p = 0.040$) in plasma relative to aPD1 therapy alone. We have shown that PGE2/COX2 pathway inhibition with ibuprofen strongly synergizes with aPD1 therapy in a murine model of melanoma, complementing existing evidence. Furthermore, combining ibuprofen with aPD1 therapy rescued half of aPD1 non-responsive YUMMER1.7 tumors and significantly extended survival in immunologically cold YUMM1.7 tumors. Interestingly, in YUMMER1.7, ibuprofen alone had superior antitumor effects to aPD1 alone and similar effects to the combination of aPD1 and ibuprofen. This suggests that PGE2/COX2 inhibitors such as NSAIDs, which are over-the-counter agents with a well-studied safety profile, may serve as a promising means of enhancing the response to aPD1 therapies such as nivolumab in melanoma patients who initially fail aPD1 monotherapy.

Keywords: Melanoma, Ibuprofen, COX2, PD1.

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## B048 / Reprogramming the tumor microenvironment by activation of p53 enhances anti-tumor effects of immune checkpoint blockade in preclinical models.

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An emerging body of literature suggests that non-cell autonomous effects of p53 can induce anti-tumor immunity. Activation and stabilization of p53 could therefore alter the tumor microenvironment, enabling more effective immune targeting of tumor cells. To explore this further, we studied the effects of enhanced p53-signaling in tumor-associated myeloid and T cells.

To evaluate the role of enhanced p53 expression in the tumor microenvironment, we used mice bearing supernumerary copies of the p53 gene (Superp53 mice) and wild-type (WT) littermate controls and implanted them with B16 (p53-WT) melanoma tumor cells. The tumor infiltrating cells in the Superp53 mice contained a greater frequency of activated CD8+ T cells with higher expression of Ki67, Granzyme-B and PD-1. The Superp53 T cells also expressed elevated levels of IFN-gamma and TNF-alpha compared with WT. Superp53 mice implanted with B16 tumors and treated with anti-PD1 antibody (RMP1-14) had delayed tumor growth compared to WT littermates ($p<0.01$) treated with anti-PD1 and controls, suggesting that enhanced p53 expression in the host augments the effects of immune checkpoint blockade. The differences in tumor control and survival were lost when T cells were depleted. To gain an in-depth understanding of the B16 tumor microenvironment in Superp53 vs. WT mice, we sorted infiltrating myeloid, CD4+ and CD8+ T cells, and studied the global transcriptome by RNASeq. We observed distinct p53-dependent gene signatures, associated with p53-dependent Rb1 and Atm1 repression, in the CD8, CD4 and myeloid cells. In myeloid cells, we found enhanced regulators of inflammation including RelA, Ccl5, TNF-alpha and ICAM-1. Accordingly, multiplexed cytokine array analysis of tumor extracts demonstrated an inflammatory cytokine profile.

We next studied the implications of pharmacologically activating p53 with APR-246. APR-246 reversibly binds the core domain of p53 through alkylation of thiol groups, shifting the equilibrium towards folded protein to reactivate endogenous p53 activity. In vitro treatment of B16 cells with APR-246 caused intracellular accumulation of p53, leading to increased apoptosis. However, treatment of B16-melanoma bearing mice with APR-246 monotherapy did not result in significant changes in tumor growth or survival. However, analyses of the tumor immune microenvironment by flow cytometry revealed increased immune potentiating M1 pro-inflammatory tumor-associated macrophages, and Ki67, Granzyme B and PD-1 in CD8+ T cells, similar to that observed in Superp53 mice. Concomitantly, there was increased PD-L1 expression in the macrophages, and Foxp3+ Tregs in tumors from APR-246 treated animals. The combination of anti-PD1 antibody

with APR-246 treatment in B16-bearing mice led to a significant delay in tumor growth ($P < 0.001$), and improved survival compared to anti-PD1 or APR-246 monotherapies ($P < 0.01$). Improved responses were also seen in MC38 colorectal cancer-bearing mice ($P < 0.01$). The anti-tumor effects of APR-246 and anti-PD1 were T cell dependent and abrogated in hosts lacking T cells. We analyzed the global transcriptome of the myeloid, CD4+ and CD8+ T cells in the microenvironment by RNAseq and found a similar pattern of inflammation with APR-246 therapy as that seen in the B16-tumor-associated myeloid cells of Superp53 mice.

Our studies support a role for enhancing the p53 effect in the tumor microenvironment and provide evidence that treatment with APR-246 can enhance anti-tumor immune responses and inhibit tumor growth in preclinical models.

Keywords: p53, APR-246.

## B049 / Complete response of stage IV pancreatic cancer combining low-dose checkpoint inhibitors with interleukin-2 (IL-2) and fever range hyperthermia

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Advanced stage inoperable pancreatic cancer has a poor prognosis and patients rarely enjoy durable complete response to treatment; progression free survival often is limited.

Materials and Methods: We previously reported several cases of complete remission of far advanced lung metastasis in triple negative breast cancer, esophageal cancer and breast cancer at ITOC3 (Munich) 2016, ITOC 4 (Prague) 2017 and ITOC 5 (Berlin) 2018 respectively; here we report a similar case.

The patient was a 45-year-old male newly diagnosed 05/2017 with adenocarcinoma of the pancreas with histological confirmation of primary invasive ductal adenocarcinoma of the pancreas with disseminated liver metastasis (>20 single lesions up to 2cm) and a single large peritoneal deposit (2.7×2.0×3.9 cm) close to the caecum. There was small volume malignant ascites. Histology revealed adenocarcinoma stage UICC IV T2 N2 M1 (hepar, peritoneum) with disseminated para-aortal and celiac lymph node metastasis. Guardant360 sequencing indicated somatic alteration burden of 9.2%. Analysis of circulating Tumor cells (CTC) revealed a high score of 236. Laboratory showed elevated transaminases and pancreatic enzymes, TM CEA/ was 3.4 ng/ml, CA19/9 4 U/ml.

The patient underwent one-time neoadjuvant CHT with Gemcitabine–Abraxane prior. Clinically the patient presented with Karnofsky index of 90% with moderate weight loss of 4 kg in the last 2 months, the patient experienced mild left upper abdominal discomfort which started around 9 months ago VAS 2-3.

Therapy consisted of administration of the following combination protocol: Low-dose PD-1 immune checkpoint (IC) inhibitor nivolumab (0.5 mg/kg) with CTLA-4 IC inhibitor ipilimumab (0.3 mg/kg) administered weekly, over three weeks. This was accompanied by loco regional hyperthermia with radiofrequency fields (13.56 MHz) using the Syncrotherm device 3 times per week (max output 400 w) over the tumor region in combination with high dose vitamin C (0.5 g/kg) and alpha lipoic acid (600mg) over three weeks. This was followed by long duration fever range whole body hyperthermia (using the Heckel HT3000 device) in combination with low dose chemotherapy using cyclophosphamide 300 mg/m² to down modulate Treg cells. Moderate dose i.v. Interleukin-2 (IL-2) under Taurolidine protection was administered for five days with careful titration to daily fever hyperthermia of max 39.5°C. CHT was administered with metronomic gemcitabine 500mg/m2 two times.

Results: First restaging 11/2017 three month following initiation of therapy with CT of abdomen and pelvis demonstrated major partial remission with decrease of the size of disseminated liver metastasis and no measurable primary pancreatic tumour, vanishing of the previously described lymphadenopathy. At that time the patient had started gaining weight again and was free of any cancer-related symptoms. Second restaging 05/2018 nine months following initiation of therapy with CT of the abdomen and indicated complete remission. Follow-up time with NED now is 2 years. Patient is healthy and free of any symptoms.

Conclusion: This is one of several cases of advanced stage cancer patients having a complete response to primary immunotherapy treatment. Clearly, this combination immune therapy warrants further clinical studies.

Keywords: Pancreatic Cancer, Hyperthermia, Checkpoint Inhibitors, Interleukin-2.

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# Microbiota in oncoimmunology

## B050 / Identifying bacterial molecules that induce gut immune responses and characterizing their protective potential against colitis-associated cancer

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Colitis-associated cancer (CAC) is a type of colorectal cancer that develops in patients with chronic intestinal inflammation associated with inflammatory bowel disease (IBD) (1). T cells play a pivotal role in enforcing intestinal homeostasis, as alterations in the number and function of T helper 17 (Th17) and T regulatory (Treg) cells contribute to the severity of IBD. Importantly, the induction of T effector cells and maintenance of intestinal homeostasis is dependent on different members of the gut microbiome (2, 3). Hence, identifying novel bacterial immunomodulatory molecules that alone could induce colonic Tregs and Th17 cells is fundamental for the development of novel therapeutic strategies to treat IBD and CAC. Therefore, we combine immunophenotyping and immunogenomics with bacterial comparative genomics and genetics to identify bacterial molecules involved in the maintenance of intestinal homeostasis and modulation of T cells responses. Previously it was shown that the probiotic strain of Escherichia coli, Nissle 1917 (EcN), can induce both Th17 and peripheral T regulatory cells (pTregs) in the colonic lamina propria (LP) of germ-free (GF) mice (4). We recently found that the ability of EcN to induce these immune cell populations was not shared by the closely related strain of E. coli, BW25113 (EcBW). Genome comParisons between these two strains identified a set of genetic differences which could be responsible for the immunomodulatory effects of EcN. Using the Lamda red recombineering system, we generated a set of EcN mutants lacking one or multiple genes unique to EcN in comParison to EcBW. To investigate their ability to promote Th17 and Treg responses, we monocolonized GF mice with either EcN wild-type or EcN mutants and immunophenotyped the gut tissue. Interestingly, we found that mice monocolonized with an EcN mutant lacking a surface molecule showed a drastic reduction of Th17 and pTreg accumulation in the colon. In addition, a mutant lacking the ability to produce a metabolite also led to reduced pTregs, while the Th17 population was not affected. To investigate these inductions further, we performed low-input RNA-seq of various colonic immune and non-immune populations from mice monocolonized with wild-type and mutant EcN. Preliminary data suggest various interesting pathways that warrant further investigation. Identification of novel bacterial immunomodulatory mechanisms will help the development of new therapeutic strategies to ameliorate IBD and CAC.

Keywords: Colitis-associated cancer, Microbiota, T regulatory cells, Bacterial molecules.

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## B051 / Regulatory considerations for gut microbiota-based cancer therapy - US FDA perspective

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Recent studies have suggested that gut microbiota may have a mechanistic impact on tumor response to cancer immunotherapy (1, 2). Transplantation of gut microbiota derived from human checkpoint inhibitor (anti-PD 1) responders to tumor-bearing mice led these mice to respond to anti-PD 1 treatment (3). These investigations appear to provide strong scientific rationale for conducting human trials using gut microbiota-based therapies to treat cancer. However, many regulatory challenges exist for this novel approach.

We searched U.S. FDA internal database for investigational drug applications (INDs) using gut microbiota for the treatment of patients with cancer. Ten such INDs have been identified, and all INDs are for Phase 1 and Phase 2 trials for different cancer indications. Four of these trials use fecal microbiota transplantation (FMT). We reviewed and compared these clinical trial protocols with respect to donor screening, FMT Methods, dose and schedule, assessment for safety and clinical outcome, and patient follow-up. We plan to present the aggregated information and discuss the following regulatory considerations for FMTs cancer trials: 1. Fecal donor screening; 2. Manufacturing process; 3. Trial design elements: a. Study population (refractory vs. early lines of therapy) b. Treatment plans i. Oral antibiotic use prior to FMT ii. FMT formulation (liquid vs. capsule) iii. Dose/schedule and route of administration iv. Coordination with other disciplines (e.g., gastroenterologist for endoscopies) c. Study evaluation i. Analyses for recipient stool microbiota pre and post FMT and immunotherapy ii. ComParison with the donor microbiota d. Safety monitoring for unpredictable behavior of commensal bacteria in immunocompromised hosts such as cancer patients (4)

Keywords: Microbiota, Fecal Microbiota Transplant, Cancer Immunotherapy, U.S. FDA Regulatory Consideration.

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## B052 / TARGETING MICROBIOTA FOR IMPROVING CANCER IMMUNOTHERAPY

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The treatment and outcome of advanced melanoma has been dramatically modified in the last decade by the development of immunotherapy and mainly immune checkpoint blockers (ICBs). Even if some patients experience long term survival, only 20% of the advanced melanoma benefit from ICBs. Recently, intestinal bacteria have been shown to impact chemotherapy and immunotherapy, including ICBs therapy [1]. However, while all the studies reported the importance of gut microbiota, they highlighted different bacterial players. Dietary manipulation represents an attractive approach of microbiome modulation to enhance response to immunotherapy. Using a preclinical model, we have demonstrated that the anti-tumor efficacy of anti-PD1 was reduced and delayed in the absence of dietary fiber. A higher percentage of Regulatory T cells has been observed in the tumor microenvironment of mice fed with a fiber-free diet. Finally, a protective association was found between Bifidobacterium abundance and tumor size, validating the recent literature[2]. Mechanistic studies are ongoing to better understand the cause-effect relationship between dietary component, gut microbiota and response to ICBs therapy. Yet, the possibility of targeting the microbiota to improve cancer treatment is an exciting one that is likely soon to become a reality.

Keywords: Cancer immunotherapy, anti-PD1, gut microbiota, Dietary fiber.

References:
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## B053 / BACTERIOPHAGE DELIVERY INTO TUMOR ASSOCIATED BACTERIA

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Introduction: Recent studies demonstrate that bacterial species are present within the tumor microenvironment (Bhatt et al., 2017). These bacteria were suggested to promote tumor growth (Rubinstein et al., 2013 & Yang et al., 2017), enable tumor cells to evade the immune system (Gur et al., 2015) and provide resistance to cancer therapeutics (Yu et al., 2017 & Geller 2017). Fusobacterium nucleatum (F. nucleatum) was shown to be significantly enriched in human colorectal carcinomas, pancreatic cancer, breast cancer, esophagus cancer, adenocarcinomas and in stomach cancer (Abed et al., 2017), compared with matched adjacent noncancerous tissue or tissue from healthy controls. A higher abundance of F. nucleatum has been associated with advanced tumor stage and poor prognosis in colorectal carcinomas (Mima et al., 2016). Bacteriophage ('phage') are viruses that specifically infect bacteria, and as such they play a critical role in regulating bacterial populations. Thus phage may serve as an anti-bacterial candidate therapy for tumor-associated bacteria. The present study aims to demonstrate phage delivery into tumor associated F. nucleatum.

Methods: Natural phage were isolated from clinical samples, sequenced and tested against F. nucleatum strains. To study in vivo phage delivery, a syngeneic subcutaneous mouse model of murine colon carcinoma tumors (CT26) was used. F. nucleatum was injected IV and survival in the tumor environment was determined by extraction and colony counts (CFU). Phage delivery into mice was carried out by a single IV administration and phage abundance in serum and tumors was determined by plaque forming units (PFU) on lawns of F. nucleatum. Phage infection of intra-tumoral F. nucleatum was measured by a qPCR designed to specifically detects infected F. nucleatum.

Results: Several novel phages for F. nucleatum were discovered and characterized for host range on clinical F. nucleatum isolates. One F. nucleatum strain and a corresponding phage were chosen for the in vivo study. Following IV administration, F. nucleatum was highly accumulated within the tumors (2x10^7 CFU/gr tumor). A pharmacokinetic study showed that, following IV administration of 10^9 pfu/mL, phage levels in serum were above 10^7 PFU/mL for at least 6 hours and were reduced after 24 hours to 10^4 PFU/mL. Phage delivery to intra-tumor F. nucleatum was demonstrated by significantly increased expression of the infected bacteria as seen by qPCR ($p<0.0001$, relative to mice that were administered with phage buffer).

Conclusion: We have shown that IV administered phage can reach tumors and infect intra-tumoral bacteria. Phage successfully delivered to target intra-tumor bacteria may be used as a candidate therapeutic approach to eradicate pathogenic tumor associated bacteria. We plan to further study phage combinations and their efficacy, together with potential combination with current therapy.

Keywords: Bacteriophage.

References:
Bhatt et al., 2017 Rubinstein et al., 2013 Yang et al., 2017 Gur et al., 2015 Yu et al., 2017 Geller 2017 Abed et al., 2017 Mima et al., 2016

## B054 / Leveraging the Second Genome platform to develop microbiome-derived cancer immunotherapies

Bernat Baeza-Raja (Second Genome, Inc).

The gut microbiota has emerged as an important player in cancer pathology and increased evidence supports its influence on clinical response to immune checkpoint inhibitor (ICI) therapy. However, the specific gut bacteria-derived molecules responsible for the improved response to ICI therapy remain unknown. Second Genome has developed a unique discovery platform to identify, screen, and validate microbiome-derived peptides that promote response to cancer-immunotherapy. Using our multi-technology meta-analysis of published datasets characterizing the baseline microbiome from melanoma patients treated with anti-PD-1, we have identified gut microbiota strains differentially abundant in responders versus non-responders that are concordant across multiple cohorts. Next, peptides from the strains associated with responder signatures were predicted from their genome sequences. In addition, we identified and selected predicted bacterially-derived peptides associated with responder signatures from assembled metagenomes. All predicted peptides were screened using phage display technology to select binders to immune cells known to play a role in the tumor microenvironment (TME). Selected microbiome-derived peptide binders were evaluated for activity in cell-based assays using isolated human primary T cells, dendritic cells, and macrophages. We show that several selected peptide binders induce secretion of proinflammatory cytokines and chemokines (e.g., IP-10, TNF) from human primary monocyte-derived dendritic cells, as well as effector cytokine secretion (e.g., IFNγ, IL-2) from human primary T lymphocytes. Additionally, we have identified microbiome-derived peptide binders with capacity to inhibit an M2-like phenotype in macrophages (decreased LPS-induced IL-10 secretion). Collectively, our results demonstrate the potential of Second Genome's discovery platform to identify and characterize novel immunomodulatory factors produced by the gut microbiota. Second Genome's discovery platform offers a unique approach to identify novel agents with potential for use as therapeutics in cancer immunotherapy.

Keywords: Microbiome, Therapeutic peptides, immunomodulation, cancer biology.

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Dhwani Haria*, Jayamary Divya Ravichandar*, Lynn Yamamoto*, Bernat Baeza Raja, Cheryl-Emiliane Chow, Joanna Dreux, Kareem Graham, Kathryn Iverson, Shoko Iwai, Sunit Jain, Yuliya Katlinskaya, Sabina Lau, Jina Lee, Michelle Lin, Paul Loriaux, Nicole Narayan, Erica Rutherford, Connor Skennerton, Thomas Weinmaier, Michi Willcoxon, Yonggan Wu, Todd DeSantis, Toshihiko Takeuchi, Karim Dabbagh, and Helena Kiefel Second Genome Inc., 341 Allerton Ave. South San Francisco, CA, 94080. *These authors contributed equally to this work.

## B055 / Investigating immune-microbiota interaction in genetically engineered mouse model of lung cancer

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The development of lung cancer is closely associated with chronic inflammation, but the source of such inflammation has not been clearly defined and the contribution of specific cellular and molecular component of the immune system is yet to be elucidated. Pulmonary infections are very common in lung cancer patients and affect clinical outcomes, but these associations are not understood in molecular details. In this manuscript, we provide strong evidence that local microbiota play a critical role in promoting lung cancer development by provoking tumor-associated inflammation.

Using an autochthonous genetically engineered mouse model (GEMM) of lung adenocarcinoma, we found lung cancer development was associated with increased total bacterial load and reduced bacterial diversity in the airway. Our data suggested that tumor-associated barrier defects and airway obstruction might result in impaired bacterial clearance and altered growth conditions for microbes. This dysregulated local microbiota stimulated IL-1β and IL-23 production from myeloid cells in a Myd88-dependent fashion, resulting in proliferation and activation of lung-resident Vγ6+Vδ1+ γδ T cells. These γδ T cells produced IL-17 to promote neutrophil infiltration and inflammation in the tumor microenvironment; they also expressed IL-22, amphiregulin and other effector molecules to directly enhance tumor cell proliferation. Mice rederived to germ-free condition or treated with combined antibiotics were profoundly protected from lung cancer initiation and progression induced by oncogenic Kras and p53 loss; on the other hand, intratracheal inoculation of bacterial mixture isolated from late-stage lung tumors robustly accelerated tumor growth in naïve animals. Bulk and single-cell RNA-seq based transcription-

al profiling further revealed that microbiota induced robust gene expression changes in a number of tumor-associated immune cell populations. In addition, we also performed 16S FISH in combination with immunofluorescence microscopy to show the direct interaction between bacterial and immune cells in the native lung tumor microenvironment. Therapeutically, we found blocking the γδ T or its downstream IL-17, IL22 signaling all significantly suppressed tumor growth in the lung. Importantly, we demonstrated that the key findings from the mouse model are closely relevant in human lung adenocarcinoma patients.

In summary, our study clearly links local microbiota-immune crosstalk to lung tumorigenesis, and thereby define key cellular and molecular mediators that may serve as effective targets in lung cancer treatment and prevention.

Keywords: microbiota, inflammation, IL-17, lung cancer.

# New trends in technology & informatics

## B056 / Investigating correlates of clinical and immune response to cancer immunotherapy using a novel unbiased cell population discovery Method in whole blood single-cell, flow cytometry

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The Cancer Immunotherapy Trials Network (CITN) coordinates multi-center cancer immunotherapy trials that are paired closely with correlative biomarker studies, wherein multiparameter flow cytometry is performed in real time on longitudinally collected whole blood samples. The purpose of this study is to describe an immune monitoring approach that combines quantitative, whole blood flow cytometry (using diverse immunophenotyping staining panels) with an automated, unbiased, cell population discovery method to enable discovery of informative correlative biomarkers.

We have recently reported clinical trial results from two CITN multi-center trials. We have also reported a non-parametric method for unbiased cell population discovery that annotates cell populations with biologically interpretable phenotypes through a new procedure called Full Annotation Using Shape-constrained Trees (FAUST). We used the unbiased FAUST procedure to analyze multi-parameter flow cytometry data generated from these two cancer immunotherapy clinical trials to demonstrate that candidate biomarkers can be associated with clinical outcome.

Here we provide comparative analyses of these flow cytometry results, performed both by conventional manual gating strategies as well as the FAUST method to highlight the value of this approach in identifying within fresh whole blood predictive biomarkers of clinical responses in immunotherapy.

In conclusion, by combining whole blood flow staining with a non-parametric method of data analyses, our results demonstrate the ability to capture important minor cell subpopulations that may not be observed when using biased manual gating that is limited to characterizing cell populations considered a-priori to be significant. In addition, our results emphasize the unique value for multi-center trials of performing flow cytometry using fresh whole blood which preserves the minor cell populations captured/identified by FAUST which may be lost or compromised by standard cryopreservation Methods.

Keywords: Correlative Biomarker Discovery, Immune Monitoring, Flow Cytometry.

## B057 / Identification of pharmacological agents that induce HMGB1 release

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The translocation of the protein high mobility group box 1 (HMGB1) from the nucleus to the cytoplasm and its secretion or passive release through the permeabilized plasma membrane, constitutes a major cellular danger signal. Extracellular HMGB1 can interact with pattern recognition receptors to stimulate pro-inflammatory and immunostimulatory pathways. Here, we developed a screening assay to identify pharmacological agents endowed with HMGB1 releasing properties. For this, we took advantage of the "retention using selective hooks" (RUSH) system in which a streptavidin-NLS3 fusion protein was used as a nuclear hook to seques-

trate streptavidin-binding peptide (SBP) fused with HMGB1 and green fluorescent protein (GFP). When combined with biotin, which competitively disrupts the interaction between streptavidin-NLS3 and HMGB1-SBP-GFP, immunogenic cell death (ICD) inducers such as anthracyclines were able to cause the nucleo-cytoplasmic translocation of HMGB1-SBP-GFP. This system, was used in a high-content screening (HCS) campaign for the identification of HMGB1 releasing agents. Hits fell into three functional categories: known ICD inducers, microtubule inhibitors and epigenetic modifiers. These agents induced ICD through a panoply of distinct mechanisms. Their effective action was confirmed by multiple Methods monitoring nuclear, cytoplasmic and extracellular HMGB1 pools, both in cultured human or murine cells, as well as in mouse plasma.

Keywords: chemotherapy, DNA methylation, HTS, immunogenic cell death.

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## B058 / Single-cell immune biomarker discovery in whole blood

Alex Chenchik (Cellecta).

New rapid and robust transcriptome-based Methods for biomarker discovery in whole blood are needed to improve diagnosis and treatment of cancer and other diseases. However, the biomarker profiling in heterogeneous blood cell populations is intrinsically limited by the fact that the readout of any pooled assay represents a “weighted average” of that population’s cellular constituents which are different for each clinical sample. Compared to conventional gene expression analysis in pooled RNA, single-cell expression analysis can reliably detect transcripts in rare cell populations that are below the threshold in bulk RNA. Nonetheless, single-cell sequencing is technically challenging due to the limited biological material in an individual cell and the high cost of sequencing across multiple cells. To address these limitations, we are developing highly scalable protocols for whole blood cell stabilization, followed by single-cell targeted multiplex RT-PCR-NGS digital expression profiling. Importantly, we developed a novel protocol for single-cell expression profiling in semi-solid media which doesn’r require special cell partitioning instrument and could applied for cost-effective analysis of thousands PBMC or FACS sorted cell fractions. This biomarker discovery platform provides at least 100-fold better sensitivity and significant improvement in sample-to-sample variability than conventional bulk expression profiling in whole blood. Also, using a defined set of amplicons and internal calibration standards significantly improves batch-to-batch variation, data analysis and sample-to-sample normalization.

We will present the performance of the genome-wide multiplex RT-PCR-NGS strategy for profiling PBMC subsets in normal whole blood samples, basally and in response to immunomodulatory stimuli ex vivo. Notably, we identify canonical cell types and determine their activation states. Preliminary studies demonstrate the assay has unparalleled specificity and sensitivity, resulting in better detection of low abundance immune biomarkers as well as an improved cost-effectiveness for high-throughput clinical applications.

Keywords: immunity biomarker, single-cell analysis, expression profiling, whole blood.

## B059 / A fully integrated platform for functional deep mining of antibody repertoires from a variety of species to secure clinical lead candidate antibodies against conserved and difficult targets

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Traditional hybridoma antibody cloning workflows are limited to rodent B cells and only sample a minority of the immune repertoire. Symphogen has pioneered a fully integrated approach for cloning, expression and functional screening of large immortalized antibody repertoires derived from millions of B cells isolated from humans, transgenic mice, transgenic rat or chickens. Our powerful methodology combines antibody cloning from single sorted B cells, Next Generation Sequencing and automated high-throughput functional screening to identify antibodies with rare binding and functional properties. Case stories showing how our approach allows for unlimited robust functional screening of 1000s of unique antigen-specific recombinant antibodies in the final therapeutic format will be presented. Combining antibody cloning from chicken B cells with high-throughput humanization, we demonstrate how chicken-derived antibodies can offer unique advantages over rodent-derived antibodies. Such advantages include access to unique functional epitopes, ultra-high affinity and importantly cross-reactivity to both non-human primate and rodent target orthologs. Cross-reactivity to rodent target orthologs enables rigorous pre-clinical evaluation of the actual lead antibody in relevant in vivo models and eliminates the need for surrogate antibodies. Sym021, an anti-PD1 antibody in clinical phase 1, is a first in man chicken-derived antibody originating from our B cell technology platform.

## B060 / Increased HLA-peptidome coverage by coupling gas-phase fractionation to LC-MS/MS

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Highly polymorphic class I human leukocyte antigen (HLA) molecules present short peptide sequences from endogenous or foreign proteins to cytotoxic T cells. Each allele is estimated to bind and present 1, 000 – 10, 000 unique peptides, but the rules of antigen presentation are not yet fully understood. Mass spectrometry (MS) holds the advantage of enabling direct identification of endogenously processed and presented peptides(1). In particular, MS detection of antigens presented by tumor cells can confirm selected epitopes, give rise to new potential antigens for therapy and monitor changes in antigen presentation in response to treatment.

Despite recent advances in instrumentation, the study of HLA peptides by mass spectrometry can still benefit from improvements in accuracy and sensitivity. Often, available material is limited and extensive sample handling leads to sample loss. Methods capable of deep profiling of HLA peptides using small amounts of tissue will be required for analysis of primary tumor samples obtained in a clinical setting. Separation of ions in the gas phase (ion mobility) can enable distinct mass analysis of otherwise interfering sets of peptides and thus, provides additional selectivity and sensitivity(2).

Here, we investigated the potential benefits of gas phase fractionation to increase the depth of the immunopeptidome coverage starting with relatively low amounts of input HLA peptide. HLA peptide complexes were immunoprecipitated from up to 50 million cells or 0.1 g of tumor material. HLA bound peptides were acid eluted, analyzed by high resolution liquid chromatography coupled tandem mass spectrometry (LC-MS/MS) and mass spectra were interpreted with the Spectrum Mill software package. This study employed a Fusion Lumos orbitrap based mass analyzer (Thermo Fisher Scientific) equipped with a FAIMS pro interface (high field asymmetric waveform ion mobility) that employs a

cylindrical electrode design to achieve gas-phase ion separation on the millisecond timescale, prior to conventional analysis in the MS/MS system. For initial evaluations, we acquired data on HLA-bound peptides eluted from three monoallelic B721.221 cell lines. When using FAIMS, the number of peptide identifications could be increased up to 40% compared to our standard workflow on a Fusion Lumos using the same input. Charge state and peptide length distribution also followed the same trends observed without ion mobility.

All newly identified peptides matched the main motif of the allele and data from the different acquisition Methods also revealed the same submotif clusters. These results demonstrate that ion mobility can sequence the HLA peptidome to a greater depth without biasing the coverage. For multiallelic samples such as tumors and patient derived cell lines, the number of identified peptides could also be increased by >30% using FAIMS.

In conclusion, coupling gas phase fractionation to LC-MS/MS can deepen the immunopeptidome coverage in a single LC-MS/MS run starting with lower sample amounts. Upfront gas phase ion separation combined with ever faster and more sensitive MS are likely to enable routine detection of less abundant peptide species such as neoantigens.

Keywords: immunopeptidomics, mass spectrometry, ion mobility, neoantigen.

References:
(1) Abelin, J. G. et al. Mass Spectrometry Profiling of HLA-Associated Peptidomes in Monoallelic Cells Enables More Accurate Epitope Prediction. Immunity 2017 (2) Hebert, A. S. et al. Comprehensive Single-Shot Proteomics with FAIMS on a Hybrid Orbitrap Mass Spectrometer. Anal Chem 2018

## B061 / Empty peptide receptive MHC class I molecules for efficient detection of antigen-specific T cells

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The peptide-dependent stability of MHC (Major histocompatibility complex) class I molecules poses a substantial challenge for their use in peptide-MHC multimer-based approaches to comprehensively analyze T cell immunity. To overcome this challenge, we demonstrate the use of functionally empty MHC class I molecules stabilized by a disulfide bond to link the alpha-1 and alpha-2 helices close to the F pocket. Peptide-loaded disulfide-stabilized HLA-A*02:01 shows complete structural overlap with wild type HLA-A*02:01. Peptide-MHC multimers prepared using disulfide-stabilized HLA A*02:01, HLA A*24:02, and H 2Kb can be used to identify antigen specific T cells, and they provide a better staining index for antigen-specific T cell detection compared to multimers prepared with wild type MHC class I molecules. Disulfide-stabilized MHC class I molecules can be loaded with peptide in the multimerized form without affecting their capacity to stain T cells. We demonstrate the value of empty loadable tetramers that are converted to antigen-specific tetramers by a single-step peptide addition through their use to identify T cells specific for mutation-derived neoantigens and other cancer-associated antigens in human melanoma.

Keywords: Empty MHC class I, MHC tetramers, T cells, Immunotherapy.

References:
Z. Hein et al., Peptide-independent stabilization of MHC class I molecules breaches cellular quality control. J. Cell Sci. 127, 2885–97 (2014)

## B062 / Opposing immune and genetic forces shape oncogenic programs in synovial sarcoma

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Synovial sarcoma is a highly aggressive neoplasm, characterized by exceptionally low T cell infiltration levels, despite high levels of immunogenic cancer testis antigens. The disease is invariably driven by the SS18-SSX oncoprotein, which leads to genome-scale dysregulation of the chromatin structure and gene transcription. To study the cancer immune interplay in this disease we developed new computational approaches and applied them to a new cohort of single-cell RNA-Sequencing and transcriptomic imaging data that we collected from 10 synovial sarcoma patients. Our data reveals that – despite their cold phenotype – synovial sarcoma tumors contain active and clonally expanded T cells, and that the dysregulation of SS18-SSX targets is coupled to oxidative metabolism and repressed immune responses in the malignant cells. Using genetic and pharmacological perturbations, we show that SS18-SSX is promoting immune resistant cell states, while cytokines produced by macrophages and T cells in the tumor microenvironment reverse the dysregulation of SS18-SSX targets. Regulatory network analyses then predicted that SS18-SSX promotes these resistant cell states through the combined activity of HDAC1 and CDK6. Confirming these predictions, HDAC and CDK4/6 inhibitors synergistically activated cell autonomous immune responses and antigen presentation specifically in synovial sarcoma cells. Taken together, our study provides new approaches to map bi-directional interactions between malignant and immune cells, and strengthens the notion that canonical oncogenes can function as master regulators of cellular immunity. Targeting the corresponding oncogenic pathways can substantially modulate cancer-immune interactions in synovial sarcoma, and potentially in other malignancies.

Keywords: Single cell analyses, Cell-cell interactions, Tumor ecosystem, T cell exclusion.

## B063 / MHC II dCODE Dextramer reagents allow single cell characterization of antigen-specificity, TCR clonotype and gene expression of CD4+ T cells

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T-cell mediated adaptive immunity to pathogens, tumor- and self-antigens in autoimmunity, is dependent on the specific recognition of the MHC-peptide (pMHC) antigen by a unique TCR on the target cell. The identification of specific T cells, their functional potential and clonotypic TCR sequence is essential to unravel the complexity of the immune responses, and to manipulate it for therapeutic benefits.

We have developed an MHC multimer technology, the dCODE Dextramer reagents, composed by a dextran backbone displaying multiple pMHCs and a DNA barcode unique for the displayed pMHC specificity. The dCODE Dextramer is compatible with 10x Genomics Chromium platform for single cell analysis.

In this study we have used a panel of MHC II dCODE Dextramer reagents to characterize antigen-specificity, TCR clonotype and gene expression of single CD4+ T cells from human peripheral blood.

Initially, the integrity of each dCODE Dextramer was demonstrated by specific labelling of CD4+ T-cells in flowcytometry. Subsequently, using the MHC II dCODE Dextramer panel we were able

to identify and correlate antigen specific CD4+ T cells, with their unique TCR clonotypes and phenotypic gene expression profiles.

In conclusion, the MHC II dCODE Dextramer can reveal 1) antigen specificity, 2) TCR clonotype and 3) functional gene expression profile of single CD4+ T cells. This allows a new understanding of cellular immunology that can progress immunological research and immunotherapeutic development.

Keywords: dCODE Dextramer Technology, TCR immune profiling, single cell analysis, NGS.

## B064 / The development of a membrane-free 'cold' and 'hot' tumor model in the high throughput compatible Mimetas' OrganoPlate® platform

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Immunotherapy is a growing field to treat tumors, where the immune system of a patient is stimulated to fight the cancer. The tumors which are sensitive to this kind of therapies are referred to as 'hot' tumors. However, resistance to immunotherapies is observed in a subset of tumors. Resistance to immunotherapy may be caused by several mechanisms, including a lack of tumor T cell infiltration caused by a lack of antigens, or a lack of antigen presentation. These resistant tumors are referred to as 'cold tumors'. A field to discover therapies to turn these 'cold' tumors 'hot' again is emerging.

Here, we describe the development of 3D co-cultures with 'hot' and 'cold' tumor models including T cells and an endothelial vessel under pump-free flow conditions in the Mimetas' OrganoPlate platform. In the development of this model the 3-lane OrganoPlate was used, which allows for membrane-free compartments mimicking lane 1) the blood vessel containing immune cells, lane 2) a compartment with tumor cells seeded in an extracellular matrix (ECM) and lane 3) a final compartment which can be used to manipulate the immune response by adding different components to the medium.

We developed two models of which one includes a matched pair of T cells and tumor cells ('hot' tumor) and one model with tumor cells lacking the antigen specifically recognized by the T cells. Tumor cells were seeded in a collagen I ECM, allowing the development of spheroids. The MHC class I expression of the tumor cells was determined by immunostaining to make sure antigen presentation was possible.Endothelial cells were seeded against the ECM and an endothelial vessel was formed after applying a pump-free flow. The endothelial vessel allows for the T cell adhesion by the expression of different selectins and integrins, needed for the migration into the ECM. Migration could be confirmed and quantified by labelling the T cells by a CellTracker. In contrast to the unpaired T cells and tumor cells, co-localization of the matched T cells and tumor cells was observed resulting in the specific killing of the tumor cells.

This new model in the Mimetas' OrganoPlate platform allows to study the effects of different components on the killing and migration capacity of CD8+ T cells. The 3-lane design is composed of 40 chips allowing for the screening of components manipulating the immune response. Therefore, this co-culture model is of high use in understanding and overcoming the resistance to current immunotherapies.

Keywords: Organ-on-a-chip, immunotherapy, hot tumor model, cold tumor model.

References:
1. Darvin P et al. Immune checkpoint inhibitors: recent progress and potential biomarkers. EMM. 2018 volume 50, 165. 2. van Duinen V et al. 96 perfusable blood vessels to study vascular permeability in vitro. Sci Rep. 2017;7(1):18071

## B065 / A 3D T cell migration model to study transendothelial migration in healthy and diseased conditions in a high-throughput microfluidic culture platform

Luuk de Haan (MIMETAS BV), Johnny Suijker (Mimetas BV), Karla Queiroz (MIMETAS BV), Erik Walinga (Mimetas BV), Henriette L Lanz (Mimetas BV), Lenie van den Broek (Mimetas BV).

Using the patients' own immune system has been shown to be a promising avenue for combatting cancer although therapeutic efficacy varies significantly depending on the tumor type. To improve this treatment and expand its use to multiple other tumor types, new models that manage to better recapitulate the in vivo processes should be developed. Here, we describe a 3D co-culture model comprising an endothelial vessel and immune cells in the high-throughput (n=40) microfluidic culture platform called OrganoPlate® that can be used to investigate the transendothelial migration of immune cells under healthy and diseased circumstances.

Endothelial cells are cultured against an extracellular matrix gel in the upper compartment of the microfluidic platform and are perfused using a rocker system resulting in the formation of a vessel. Upon formation of a vessel with high barrier integrity, the vessel is perfused with medium containing T cells. The bottom perfusion lane of the microfluidic chip is used to add triggers and stimulate T cell migration.

Formation of vessels with high barrier integrity is observed within 6 days after seeding endothelial cells as confirmed by an assay using cell-impermeable fluorescent dyes. High barrier integrity was retained for another 48 hours of co-culture with T cells. Upon their addition, T cells are seen to slow down and attach to the endothelial vessel wall, as confirmed by immunofluorescent staining. T cell numbers in the endothelial vessel are seen to increase over time, showing a significant difference between unstimulated and stimulated T cells. The occurrence of the process of transendothelial migration can be affected by addition of triggers like chemokines. The extent to which this happens, however, is highly dependent on the integrity of the endothelial barrier as T cells are observed to migrate to a lesser degree when the barrier is intact whereas migration increases when barrier integrity is lowered, something that occurs in vivo in case of inflammation.

This co-culture model allows one to follow the rolling, tight adhesion and subsequent transendothelial migration of T cells, which are key processes of immunotherapy, in healthy and disease states in a real-time manner. Furthermore, the bottom compartment of the microfluidic platform can be utilized to add triggers or immunotherapeutic compounds to the system and assess their effects on the migratory behavior of the T cells. Thus, this novel model presents new possibilities to improve and expand our knowledge regarding the process of transendothelial migration of immune cells during immunotherapy.

Keywords: Organ-on-a-chip, transendothelial migration, immunotherapyA 3D T CELL MIGRATION MODEL TO STUDY TRANSENDOTHELIAL MIGRATION IN HEALTHY AND DISEASED CONDITIONS IN A HIGH-THROUGHPUT MICROFLUIDIC CULTURE PLATFORM.

References:
1. Darvin P et al. Immune checkpoint inhibitors: recent progress and potential biomarkers. EMM. 2018 volume 50, 165. 2. van Duinen V et al. 96 perfusable blood vessels to study vascular permeability in vitro. Sci Rep. 2017;7(1):18071

## B066 / Combining ion beam imaging modalities for probing the T cell and B cell synapse

Maxim Markovic (Stanford University), Bokai Zhu (Stanford University), Yunhao Bai (Stanford University), Xavier Rovira-Clave (Stanford University), Sizun Jiang (Stanford University), Garry Philip Nolan (Stanford University).

Multiplexed Ion Beam Imaging (MIBI) overcomes the limitations of fluorescence spectral overlap in traditional microscopy with the use of mass spectrometry to 'read' tissue samples in two dimensions. In addition to biologically-ubiquitous elements (such as carbon, iron and phosphorus), specific antibody-conjugated

metal ions can also be detected (Angelo et al., 2014). Tissue samples stained with a cocktail of metal-conjugated antibodies can be visually reproduced in two dimensions, to allow for the highly parameterized study and analysis of human tissue samples with more than 40 markers on a single sample simultaneously. More recently, our lab described super-resolution Ion Beam Imaging (srIBI), a related method capable of reconstructing single cells in three-dimensions, with up to 7 parameters at high resolutions (as low as 50nm X-Y) (Rovira-Clave and Jiang et al., 2019). Here, we aim to overcome the technical challenges in combined analysis with both the MIBI and srIBI Methods. This will establish a novel technique for the experimental validation of cellular neighborhoods and the fine details of cell-to-cell interactions.

As a proof of concept, we first perform preliminary MIBI analysis of human tonsil samples for a multi-parametric reconstruction of germinal centers at a lower resolution (~500nm X-Y). This allows for the rapid identification of cellular interactions between cognate mature T follicular helper (Tfh) cells and naïve B cells. We then utilize srIBI to probe in detail these specific T cell and B cell synapses at an unprecedented scale. A deeper understanding of this critical adaptive immune axis will be important for understanding the full potential of adaptive cell immunotherapies.

Keywords: Super-resolution imaging, Neighborhood analysis, Immune synapses.

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## B067 / Molecular dissection of the lung cancer tumor microenvironment using spatially resolved protein/RNA quantification

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Understanding immune and stromal biology in the tumor microenvironment (TME) is critical to improving therapies and identifying new targets. Immunohistochemistry (IHC) is widely used to analyze protein abundance in biopsies with single cell resolution, but is limited in multiplex capacity. RNA-Seq Methods provide high multiplexing, but eliminate spatial resolution. Technologies to bridge the spatial resolution of IHC to the high multiplexing of transcriptomics are needed to elucidate complex tumor biology. To address this, we employed an emerging technology, Nanostring Digital Spatial Profiling (DSP) on formalin-fixed paraffin-embedded (FFPE) human lung cancer samples (4 squamous cell carcinoma, 4 adenocarcinoma [Tristar]) to profile the TME by both RNA and protein expression in two-dimensional space. Samples were sectioned and stained with a 3-color IHC for pan-cytokeratin (tumor marker), CD8 (T-cell marker), PD-L1, and a nuclear stain to guide region selection. Samples were also stained with a cocktail of 43 primary antibodies or 93 RNA probes. Tissue sections were imaged and 200-um diameter regions of interest (ROIs) were profiled using the DSP instrument. ROIs were selected using immunofluorescence (IF) as a guide for molecular dissection of the TME, according to one of the following strategies based on CD8 levels: within the tumor, on the tumor margin in healthy lung, and in tumor sections without CD8. RNA, protein counts, and digitized IF data from 456 ROIs were integrated and analyzed using linear modeling (CellProfiler [Broad Institute] and LIMMA package in R). ROIs taken from serial sections had a high degree of correlation in both RNA and protein panels, demonstrating the precision of the DSP sampling platform. Additionally, the DSP protein quantification correlated well with IF and chromogenic staining values for 5 antibody markers. Unbiased high-dimensional clustering on the protein and RNA panel showed ROIs grouped primarily by patient; ROIs in tumors were strongly differentiated from infiltrate or normal lung tissue regions. The small sample size limited the statistical power to identify differences in ROIs across patients. Nonetheless, several targets where wide expression differences were expected were readily identifiable, including beta-catenin and cytokeratin being enriched in tumor cells compared with tumor-infiltrating CD8 T cells that had elevated CD8, VISTA, and OX40L protein. Elevated levels of CD14 and HLA-DR protein were found in normal lung compared with tumor, consistent with an expected high number of alveolar macrophages in healthy lung vs tumor samples. ComParison of RNA between ROIs identified stroma enriched for ICAM1 and keratin, while infiltrate on the margin had elevated CD45 and HLA-DR. The DSP platform has high inter-section reproducibility and a good correlation between staining intensity by traditional Methods and protein quantification in the multiplexed assay. Variation between patients was the major driver of both protein and RNA expression differences, suggesting the need for a large number of patient samples to support meaningful results in human tumors. Mouse tumor models may be more homogeneous, thus requiring fewer samples. Despite this heterogeneity, strong differentials between ROIs were still detectable, suggesting the value of this platform for de novo target identification or enhanced mechanistic understanding of the spatial proteomic and transcriptomic relationships between cells within the tumor.

Keywords: Digital Spatial Profiling, Spatial Transcriptomics, Spatial Proteomics.

## B068 / Codex: A novel platform for spatially-resolved deep antigen profiling of single cells in tissue samples

Ivan Masetto (Akoya Biosciences).

Spatially resolved, deep biomarker profiling of tissue samples is crucial for investigating the architecture and cell diversity of complex matrices, such as secondary lymphoid tissues. The correlation of these parameters with the progression of different pathologies, ranging from autoimmune diseases to cancer, is still unknown due to the lack of techniques enabling detection of both high content and spatial resolution. Current methodologies for analyzing the spatial dimension of tissues, as traditional immunohistochemistry, are limited to measuring a few parameters, thereby restricting the number of identifiable phenotypes. Conversely, single-cell technologies like mass cytometry and Next Generation Sequencing tools provide multiplexing capabilities but lack any spatial information.

Akoya Biosciences is commercializing CODEX® (CO-Detection by indEXing), a multi-parameteric imaging platform that detects tens of biomarkers with single-cell resolution in a tissue specimen. CODEX uses a DNA-based barcode library to label antibodies and iterative cycles of adding and removing cognate dye-labeled oligonucleotides to reveal the staining of three markers per cycle. Data acquisition is fully automated by the CODEX instrument, which integrates with existing microscopes. The analysis pipeline includes drift compensation, deconvolution and segmentation to measure integrated fluorescence intensity for each cell across tens of parameters. Highly dimensional data can then be analyzed by an unsupervised clustering algorithm and visualized by the CODE Viewer.

For high-multiplexing capabilities, more than 80 CODEX antibodies were validated for analysis of fresh-frozen and FFPE tissues. Preliminary studies demonstrate CODEX unprecedented capability for revealing the spatial correlation among different populations of immune cells within the tissue microenvironment.

Keywords: High-Multiplexing, New technology.

References:
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## B069 / Characterization of a 582-protein panel in depleted plasma from subjects with NSCLC using data independent acquisition mass spectrometry

Nicholas Dupuis (Biognosys AG), Linda Sensbach (Biognosys AG), Sebastian Müller (Biognosys AG), Lukas Reiter (Biognosys AG).

Measurement of circulating biomarkers in cancer has proven utility for early detection, differential diagnosis, and predicting pre-treatment response to therapy. More recently, circulating proteomic biomarkers for pre-treatment prediction of therapeutic response or surrogate endpoints of response have received additional attention due to the heterogeneous responses to immunotherapies. To develop a greater understanding of the circulating plasma proteome in subjects with cancer we have optimized a depleted plasma proteomic workflow, based on data independent acquisition (DIA) mass spectrometry, combined with a panel of stable isotope labeled (SIS) peptides and applied it to plasma from subjects with late stage NSCLC. This approach provides a deep and accurate quantification of plasma proteins associated with lung cancer.

Plasma samples from subjects with Stage III-IV non-small cell lung cancer (NSCLC, n = 15) and age matched healthy donors (n = 15) were depleted of 14 high abundance proteins using MARS Hu-14 spin columns (Agilent). The resulting flow through was prepared for mass spectrometry using a filter aided sample preparation method (FASP) and just prior to analysis SIS peptides covering 582 proteins were spiked into all samples. All samples were analyzed using two-hour gradients on a nano-flow LC-MS/MS setup operated in data independent acquisition mode. Data was extracted using Spectronaut (Biognosys) with a panel specific spectral library and statistical analysis was conducted to identify disease associated biomarker candidates.

Across all samples 468 proteins were quantified with an average of 406 proteins quantified per sample. Univariate statistical testing identified 41 dysregulated proteins (28 up-regulated and 13 down-regulated; q-value > 0.05 and log2 fold change > 0.58). The protein candidates associated with NSCLC include both positive and negative acute phase reactants (e.g. Positive: CRP, HP, ORM1/2 and SERPIN, Negative: TF), complement system proteins (C1QC, C4BPa/b) and the S100 family of proteins (S100A8, A9, A12 and S100P). Additionally, LDHA, a protein with potential diagnostic and prognostic significance was found to be enriched in the NSCLC plasma. Consistent with expectation, there is an overall activation of the acute phase response, indicative of the host immune response to the tumor.

The SIS peptide panel covering a broad range of proteins, combined with the depleted plasma workflow, enables accurate quantification of many plasma proteins that are hallmarks of activated host immune responses via acute phase response signaling, innate immune response (complement system), and other proinflammatory stimuli, which have been linked to poor patient prognosis and outcomes with immunotherapies.

Keywords: Biomarkers, proteomics, blood, NSCLC.

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## B070 / Vasculature and T cell co-culture in a high-throughput microfluidic platform

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Recent clinical success of immune checkpoint inhibitors and chimeric antigen receptor T cells has highly increased the attention for the field of immunotherapy. However, identifying responders to these therapies is challenging underscoring the necessity for translational models that increase understanding of tumor-immune responses.

In the present study, a co-culture system containing immune cells and vasculature was established. Both are essential components of the tumor microenvironment and very often lacking in in vitro tumor models, highlighting the added value of our co-culture platform. We focused on optimizing endothelial and CD8+ T cell co-cultures and subsequently assessing T cell migration from the endothelial tubes via endothelial sprouts towards various chemoattractants. In order to generate stratified 3D co-cultures, the Mimetas OrganoPlate Graft containing 64 microfluidic culture units was used. The microfluidic units in this platform are composed of two parallel microfluidics channels and a central chamber. The two parallel microfluidic channels were used for generating parallel endothelial tubules, whilst angiogenic factors (S1P, VEGF, bFGF and PMA) were added to the central chamber of the culture unit resulting in a generation of a gradient and sprouting of the endothelial tubes towards the central chamber. Generated sprouts were stable and perfusable. The central chamber is designed for culturing complex microtissues such as spheroids, organoids and explants.

Angiogenic endothelial tubules formed vascular beds in presence of added factors within 3-5 days. Once vascular beds were formed, activated and fluorescently labeled CD8+ T cells were loaded in the endothelial tubules and followed in culture for 48 hours. CD8+ T cell migration was observed both via the sprouts as well as by crossing the endothelial barrier, and increased in presence of gradients of CCL2, CCXl12 and CCL9. Highest CD8+ T cell numbers were observed in presence of a gradient generated with a mix of these three chemokines.

Therefore, we present a high throughput co-culture system containing angiogenic endothelial tubules and CD8+ T cells. These co-cultures are highly suitable for studying T cell migration, event which precedes the detection and recognition of antigens at the surface of antigen-presenting cells and for interactions with other cells involved in the immune response. In addition, these co-cultures serve as a platform for understanding the interplay between T cell migration and angiogenesis in the tumor microenvironment. Furthermore, we envision that this model will evolve into an immunocompetent patient-derived tumor model that can be used to study immune responses to tumors.

Keywords: T Cell migration, Angiogenesis, 3D cell culture, Organ-on-a-chip technology.

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# Other

## B071 / Elucidation of feedback and other mechanisms of IgH allelic exclusion for production of therapeutic bispecific antibodies in mice

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Antibody genes are assembled in developing B lymphocytes from a set of gene segment cassettes known as Variable (V) diversity (D) and joining (J) segments by a process termed V(D)J recombination. The ability to fight off almost any foreign pathogen relies on the capacity of an individual to generate a nearly limitless number of different antibodies through the genetic diversification mechanisms built into the V(D)J recombination reaction. However, ability to generate antibody repertoires large enough to recognize any given pathogens comes with the risk that many of these essentially randomly generated antibodies may recognize our own antigenic molecules and cause auto-immune reactions. To combat this potentially negative impact of antibody diversification, we have evolved mechanisms to recognize and eliminate B lymphocytes that make antibodies against our own antigens. To ensure ability to accurately eliminate the "bad" auto-reactive B lymphocytes versus the "good" non-auto-reactive B lymphocytes that make antibodies against foreign pathogens, a process known as allelic exclusion restricts any given B lymphocyte to expression of just one species of antibody. Defects in allelic exclusion have been implicated in consequences ranging from auto-immunity to cancer. Despite decades of great interest and substantial investigation, the overall mechanisms that lead to allelic exclusion of antibody gene expression are still debated. My recent data implicates a potentially new component of the allelic exclusion mechanisms that functions to enforce this process even in the absence of mechanisms currently considered to serve that role. We did not observe allelic inclusion in bone marrow and splenic B cells in which feedback regulation of IgH V(D)J rearrangement was disrupted. In order to reduce the Background competition from mouse VHs, we are generating a rearranging mouse model with only the single human VH. Based on a conditional expression system, we also are developing mouse models that will produce human bi-specific antibodies in mice. This work could contribute to a fundamental understanding both of normal antibody repertoire development and mechanisms by which impairments in allelic exclusion may contribute to autoimmunity and B cell cancers. My work also could provide insights and models that could advance new in vivo approaches to generate antibody therapeutics for cancer and other diseases.

Keywords: Allelic Exclusion, V(D)J rearrangement, Bispecific Antibodies.

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## B072 / Molecular control of B cell division in Germinal Centers

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Germinal centers (GCs) emerge in secondary lymphoid organs during normal immune responses to enable proliferation, mutation and selection of B cells. These processes are essential for the evolution of protective antibody responses, but may also lead to the development of lymphomas. To better understand these outcomes, we are currently investigating two alternate modes of B cell proliferation in GCs: one that is directly dependent on T cell help and antigen-related signals, and one that is resilient to inhibition of several signaling pathways and takes place exclusively in the dark zone of GCs, in the absence of antigen or T cell help, and more closely related to lymphomas, thus named "inertial" cycles. Our current data supports the notion that "inertial" cycles require that B cells are previously "fueled" by T cell help through mTORC1 activation, and are specifically controlled by cyclin D3, whereas the signal-dependent mode of division does not rely on cyclin D3.

Keywords: germinal center, lymphoma, B cell, cyclin.

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Schmitz R, Young RM, Ceribelli M, Jhavar S, Xiao W, et al. (2012) Burkitt lymphoma pathogenesis and therapeutic targets from structural and functional genomics. Nature 490: 116-120. 1. Ersching J, Efeyan A, Mesin L, Jacobsen JT, Pasqual G, et al. (2017) Germinal center selection and affinity maturation require dynamic regulation of mTORC1. Immunity 46: 1045-1058.

## B073 / THERAPEUTIC IMPLICATIONS OF ALTERED EPIGENETICS AND DNA DAMAGE RESPONSES IN IDH2-MUTATED HEMATOLOGIC DISEASES

Julie Leca (The Campbell Family Institute for Breast Cancer Research - University Health Network).

Acute myeloid leukemia (AML) and angioimmunoblastic T cell lymphoma (AITL), are hematologic diseases requiring novel approaches to patient selection and therapy. In AITL, neoplastic cells express many markers of T follicular helper (TFH) cells, while in AML, myeloid stem and progenitor cells are affected. Tumor cells of AML and AITL patients frequently bear mutations affecting genes involved in epigenetic regulation, including isocitrate dehydrogenase (IDH) and ten-eleven translocation-2 (TET2). IDH mutations drive production of the rare metabolite D-2-hydroxyglutarate (2HG), which competitively inhibits α-ketoglutarate (α-KG)-dependent dioxygenases such as the TET proteins (affecting DNA methylation) and Jumonji histone demethylases (altering histone methylation). IDH mutations occur in 30% of AML and AITL cases but the spectrum of these mutations differs. In AML, IDH1R132 and IDH2R140 dominate, and IDH and TET2 mutations are mutually exclusive. In AITL, IDH2R172 dominate (no IDH1 mutations), with co-mutation of TET2 in 82.1% of cases.

In AML, loss of these dioxygenase activities causes epigenetic alterations and leads to abnormal gene transcription that affects hematopoietic cell differentiation, and drives myeloid disease. However, in AITL disease, the impact of the IDH2 mutation is completely unknown.

My goal is to dissect the effects of IDH2 and TET2 mutations in hematologic diseases to understand how collaborate IDH2 and TET2 mutations to drive malignancy in AITL, and why do they cooperate differently in myeloid and lymphoid diseases. I focus on DDR signaling and epigenetics analysis to found novel therapeutic vulnerabilities arise from the altered epigenetic regulation and DDR linked to IDH2 and TET2 mutations. I used a CD4-Cre mouse model to drive the IDH2R172K and TET2 mutations in the T cell compartment. Mice bearing both IDH2 and TET2 mutations show decreased survival accompanied by splenomegaly and lymphadenopathy. This is significantly different than CD4+ control mice or single mutant mice. Double mutant mice (DM) present a disruption of spleen and lymph node architecture. To understand this phenotype, I performed flow cytometry staining and found that DM mice present a T cell phenotype including a decrease of CD4+ naïve cells and an increase of CD4+ effector memory cells. So, there is an imbalance in T cell homeostasis, but only when both the IDH2 and TET2 mutations are present, suggesting a cooperative role for both mutations in T cell development.

To further explore the requirement of TCR signaling for proliferation and activation of naives CD4 cells, I studied their response to treatment with increasing concentrations of anti-CD3 antibody plus CD28. In these in vitro experiments, I found that naïve CD4 cells from DM mice proliferate slowly compare to others genotype and this is correlated with their activation statue which is also decrease. We know that others signaling can drive the T cell proliferation in AITL context, like ICOS signaling, as described by Cortes et al. in Tet2–/– RHOA G17V-expressing AITL-like tumor cells (Cortes, 2018, Cancer Cell). Most important for us, it's to check the ability of TFH polarization by these cells.

Since both IDH2 and TET2 affect epigenetic regulation, I collect TFH cells to conduct experiments to understand how these changes modulate T cells homeostasis. Moreover, emerging data support the hypothesis that connections exist between epigenetic regulators and DDR signaling in hematological diseases.

My final aim will be to attempt to evaluate the efficacy of treatment with IDH2 inhibitors, hypomethylating agents, or DDR-targeting drugs, alone or in combination in AITL disease and identify factors involved in responses to these therapies or in the development of resistance. Finally, I will compare results obtained in AML versus AITL mouse models and clinical samples to identify mechanisms that are shared, and those that are unique to each disease.

Keywords: IDH2, Lymphoma, T cells.

References:
Cortes, 2018, Cancer Cell

## B074 / Evaluation of T-cell assays performance between laboratories worldwide

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Accurate and reproducible identification of antigen-specific T cell responses are essential in immunotherapeutic research and development. Harmonization efforts by the CIC and CIMT along with the development of improved reagents and protocols have enabled improved detection by MHC multimer and T-cell Elispot assays.

Supported by CIC and CIMT, Immudex has conducted proficiency panels for MHC multimer and Elispot assays in 2019. Participation in the panels was open to any laboratory that routinely monitor antigen-specific T cell responses with either MHC multimer or Elispot assays. All participants received two pre-tested PBMC donor samples and assay instructions.

18 laboratories from 8 countries participated in the MHC multimer panel. The MHC multimer consisted of two CMV, one FLU, and one EBV epitope. The percentage of CMV-, FLU- and EBV-specific T cells for both donor samples were reported. All analyses were performed by flow cytometry. Approximately 90% of the participants' results for all measurements were in the average or near the average range.

39 laboratories from 13 countries participated in the T-cell Elispot Panel. The Elispot consisted of two predefined peptide pools, human CMV and human mixed viruses. The number of spots for each peptide pool were reported for both donor samples. Approximately 65% of the Elispot participants' results for all measurements were in the average or near the average range.

All participating laboratories showed good reproducibility between their replicates for the individual samples and every laboratory was able to find positive events in the known positive samples. Overall, the proficiency panel is a valuable tool to evaluate performance between laboratories worldwide.

Keywords: MHC multimer, Elispot, Immune monitoring, Proficiency Panel.

## B076 / Deconvoluting global proinflammatory cytokine signaling networks in natural killer cells

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Signal transducer and activator of transcription (STAT) proteins are evolutionarily conserved mediators that play critical roles in developmental and host defense pathways in the immune system. Within the natural killer (NK) cell compartment, early proinflammatory cytokine signaling mediated through STAT proteins plays a key role in promoting optimal antiviral responses. Previous and current work in the lab have described the non-redundant requirements of STAT1, STAT4, and STAT5 signaling in NK cell responses to mouse cytomegalovirus infection. Activation of these STATs are thought to be induced by distinct cytokines, i.e., STAT1 by type I IFNa, STAT4 by IL-12, and STAT5 by IL-2 and IL-15. However, the pleiotropic effects of both cytokine and STAT signaling preclude the ability to precisely attribute molecular changes to a respective cytokine and/or STAT protein. Furthermore, NK cells are exposed to all of these cytokines concurrently during infection, but we have yet to fully understand how these global proinflammatory signaling networks interact and contribute to the net effect of optimal survival, proliferation, and function. In this study, we aimed to characterize these interactions in NK cells by using a global multi-faceted approach, incorporating several high-throughput sequencing Methods (RNA-seq, ATAC-seq, and ChIP-seq) that broadly interrogate both the transcriptional and epigenetic landscapes. We uncover an IL-2/15 mediated axis that preferentially overlaps with an IL-12/18 network and overall enhances IL-12/18-induced changes. These changes are reflected on both the levels of mRNA as well as chromatin accessibility, and are distinct from the mode of regulation observed in IFNa-STAT1 mediated changes. By incorporating the global transcription factor binding profiles of these STAT proteins in response to their respective proinflammatory cytokine, we have generated a network of direct transcriptional targets that include both distinct and shared modules across all three signaling pathways. Overall, our study sheds light on the crosstalk between proinflammatory cytokine signaling pathways in NK cells and will serve as a valuable resource for improving NK cell immunotherapy.

Keywords: natural killer cells, proinflammatory cytokine, STAT.

## B077 / Tissue determinants of human NK cell development, function and residence

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Immune responses in diverse tissue sites are critical for protective immunity and immune homeostasis. Natural killer (NK) cells are innate immune cells that are important for anti-viral and tumor immunity. They can express multiple activating and inhibitory receptors in addition to the low-affinity low-affinity immunoglobulin G receptor CD16 and can kill tumor cells without prior exposure. We now know that NK cells not only direct kill tumor cells but can also mediate cancer immunosurveillance and prevent metastasis. However, at present the distribution, diversity and tissue driven differences in NK cell function are not well characterized. Here, we investigated how tissue localization regulates the development and function of human NK cells by integrating high dimensional analysis of NK cells from blood, lymphoid organs and mucosal tissue sites from 48 individuals. We identify tissue-specific patterns of NK cell subset distribution, maturation and function across age and genetic diversity. NK cells comprise a sizeable fraction of the lymphocyte compartment in blood, bone marrow, spleen and lung, where they are predominantly CD56dim, while significantly lower frequencies of NK cells in lymph nodes, tonsils and intestines are mostly CD56bright. This tissue-intrinsic profile of NK cell frequency, subset distribution and differentiation is stable across all ages and genetic diversity. Notably, precursor and immature NK cells in the lymph nodes and intestines exhibit tissue-resident molecular signatures and site-specific properties, while mature and terminally differentiated NK cells have similar effector function and shared gene expression profiles between sites. Our findings reveal tissue-mediated segregation of NK cell development and function and provide a blueprint for how NK cells are seeded, maintained and surveil tissue sites across the body. The unique phenotypic, functional and transcriptional features of tissue NK cells could be targeted in future generations of NK cell mediated immunotherapies.

Keywords: NK cells, Tissue-specific, Residence, Subsets.

## B078 / Deciphering the role of myeloid cells in inducing distinct T cell responses to gut microbes

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Gut microbial commensals and pathogens were shown to induce specific T cell programs that are mediated by antigen presenting cells (APC). APCs are separated by their ontogeny, residence and transcription factor dependency and it was suggested that the different subsets have distinct expertise in mediating immune programs and T cell polarization. Currently the exact contribution of APC subsets is still under debate and it is not clear if distinct T cell responses utilize specific APC subsets. To tackle this question, we used two established microbial models in lab. Segmented filamentous bacteria (SFB) induces a specific Th17 response in the small intestine while Helicobacter hepaticus (Hh) promotes a specific Treg response in the large intestine. Both T cell responses initiate in the mesenteric lymph node (mLN) where master transcription factors that dictate polarization are upregulated prior to T cell proliferation. Thus, we first aimed to identify the APC subset that mediates the T cell response to SFB and Hh in the mLN. Using single cell RNAseq, ex-vivo assays and photo-activation approaches, we found that migratory cDC2s present the SFB and Hh peptides as well as reside adjacent to primed T cells for both models. We are currently seeking to define different 'states' of migratory cDC2s and their potential distinct role in T cell polarization. Using genetic tools in which we prevent dendritic cells from migrating from resident tissue to lymph nodes, we found that migratory dendritic cells are crucial for Treg polarization and tolerance. Interestingly, unlike the SFB model, T cell priming to Hh was not abolished but rather enhanced and shifted toward Th17. Currently we are working to further elucidate the role of APCs that mediate this altered response. Overall, this study aims to expand our understanding of the role of APCs in balancing tolerance and inflammation in the gut.

Keywords: Antigen Presenting cells, mucosal immunology, microbiome, polarization.

## B079 / Microwave ablation enhances tumor-specific immune response in patients with hepatocellular carcinoma

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Thermal ablative therapies are standard treatments for localized hepatocellular carcinoma (HCC). In addition to local tumor destruction, ablation leads to abscopal effects in distant lesions most likely mediated by an anti-tumor immune response. Although microwave ablation (MWA) is increasingly substituting other ablative techniques, its systemic immunostimulatory effects are poorly studied. We analyzed tumor-specific immune responses in peripheral blood of HCC patients after thermal ablation with regard to T cell responses and disease outcome. While comprehensive flow cytometric analyses in sequential samples of a prospective patient cohort (n=23) demonstrated only moderate effects of MWA on circulating immune cell subsets, fluorospot analyses of specific T cell responses against seven tumor-specific antigens revealed de-novo or enhanced tumor-specific immune responses in 30% of these patients. This anti-tumor immune response was related to tumor control as Interferon-y and Interleukin-5 T cell responses against cancer testis antigens (CTA) were more frequent in patients with a long-time remission (>one year) after MWA (7/16) compared to patients suffering from an early relapse (0/13 patients) and presence of tumor-specific T cell response (IFN-y and/or IL-5) was associated to longer progression-free survival (15.0 vs.10.0 months). Digital image analysis of immunohistochemically stained archival HCC samples (n=18) of patients receiving combined ablation and resection revealed a superior disease-free survival of patients with a high T cell infiltration at the time of thermal ablation (37.4 vs.13.1 months). Our data demonstrates remarkable immune-related effects of MWA in HCC patients and provides additional evidence for a combination of local ablation and immunotherapy in this challenging disease.

Keywords: microwave ablation, hepatocellular carcinoma, immune response, immune cell subsets.

## B080 / Solubility of Folded Protein Domains Influences Formation of Biomolecular Condensates

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Biomolecular condensates have recently emerged as a broad phenomenon to describe cellular organization in the absence of a bounding membrane1. Although progress has been made in describing these condensates, their role in cellular function and disease, independent of their underlying biochemistry, is largely unknown. Devising ways to enable rational manipulation of condensates in a minimally perturbative manner would allow the possibility of manipulating cellular organization as a means of treating disease. I hypothesize that surface residues of folded protein domains can be altered to modulate their intrinsic solubility in solution without altering canonical biochemical functions. I have applied this approach to a laboratory system based on multivalent interactions between the small ubiquitin-like modifier (polySUMO) and the SUMO interaction motif (polySIM) to mimic a nuclear body that's formed from the promyelocytic leukemia protein (PML-NB)2. The solubility of an individual SUMO domain is predictive of the phase separation propensity of polySUMO with polySIM. The solubility of each domain may be altered either through solution pH or site-directed mutation on the protein surface. These perturbations do not alter the structure of biochemical interactions of SUMO. Preliminary images of PML-NB in cells show that there is a small dependence of the number of PML nuclear bodies to buffer pH, suggesting that the solubility of SUMO is a determinant of the formation of PML nuclear bodies. This work lays down the foundation for the manipulation of biomolecular condensates, especially those involved in endogenous and aberrant cellular signaling.

Keywords: Phase Separation, Cellular Biophysics, Cellular Signaling.

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## B081 / Antiviral protein viperin inhibits translation to restrict viral replication

Jack Hsu (Yale University), Maudry Laurent-Rolle (Yale University), Peter Cresswell (Yale University).

The innate immune response induces hundreds of interferon-stimulated genes (ISGs), many of which play an important role in antiviral immunity. Viperin is one such antiviral ISG, which restricts the replication of a broad spectrum of DNA and RNA viruses. However, a general mechanism for viperin action and its potential roles in innate immunity remains to be defined. Here, we report a novel antiviral mechanism for viperin, whereby viperin inhibits the translation of viral RNA. We show that viperin interacts with the translation machinery. We demonstrate that viperin inhibits global translation in the type I interferon response and in a variety of mammalian cells primarily through its radical SAM enzymatic activity. We show that viperin inhibits global translation by activating the eIF2 signaling pathway through an eIF2α kinase GCN2. Given the dependence of viruses on the host cellular machinery for translation, consequently, viperin inhibits viral protein synthesis and viral replication of Zika virus and Kunjin virus. Our study illustrates translational regulation as a key step in the antiviral response and presents viperin as the "translational regulator" in innate immunity.

Keywords: Viperin, Translational regulation, Antiviral response, Zika virus.

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## B082 / Membrane topology and patch-like receptor organization underlie T cell antigen detection

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T cells are capable of quickly and precisely identifying rare cognate antigen on target cells. T cells do this via fine membrane protrusions known as microvilli, which effectively probe opposing surfaces in search of cognate antigen. Upon ligand detection, T cell receptors (TCRs) form micro-clusters that occupy and move with microvilli in the immunological synapse (IS). It is yet unclear how TCRs, the key receptors for antigen discrimination and signal transduction, are distributed on resting T cells beyond that they do not appear to form aggregates at the molecular scale. It is also unclear how they come to occupy microvilli during activation and whether pre-assembly of co-stimulatory and co-inhibitory receptors with TCRs prior to activation are required for signaling. In this study, we use Lattice Light Sheet Microscopy to simultaneously map membrane topology and receptor distribution on resting T cells in 3D with high precision. Our results confirm earlier studies suggesting that TCRs are non-homogenously distributed into high-density patches on the membranes of effector T cells prior to activation. These patch-like distributions are highly dynamic. Although at each time point, some of these TCR patches are on microvilli tips, the localization of TCR patches is not restricted to microvilli. We also observe some microvilli with no apparent TCR accumulation. TCR patches typically move independently of microvilli or transiently associate with microvilli with t1/2 of about 6 s. These results show that TCR patches dynamically circulate in and out of microvilli during surface scanning. TCR patches are "stabilized" on microvilli only when they encounter activating peptide major histocompatibility complexes (pMHCs). Other membrane receptors, i.e. CD28, PD-1, CD8 and LFA-1, also form high-density patches. While CD8 patches show high level of colocalization with TCR patches, other receptor patches do not necessarily colocalize with TCR patches, consistent with these receptors not being pre-packaged into "signaling" units on resting T cells. During T cell and antigen presenting cell (APC) interactions, we observe T cell microvilli projecting deep into 3D pockets formed by veil structures on the surface of dendritic cells (DC). DC membrane also conforms to accommodate T cell microvilli, which creates a tight fit between the T cell and DC, increasing the effective close-contact area by as much as 100 times. Importantly, such membrane interactions between APC and T cell suggest that TCR patches do not necessarily need to be on microvilli tips to bind to pMHCs. This work demonstrates how T cell orchestrates its membrane topology and receptors during the process of antigen detection and activation.

Keywords: T cell receptor, Microvilli, Lattice light sheet microscopy.

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## B083 / Oncolytic adenovirus 3 coding for CD40L as a potent facilitator of dendritic cell therapy

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Dendritic Cell (DC) therapy is considered as a promising immunotherapeutic approach for treatment of advanced cancer. However, the tumor microenvironment is highly immunosuppressive that leads to DCs dysfunction and thus, these tumor-associated DCs indirectly support tumor progression. Therefore, in clinical trials DC therapy has generally failed to fulfill its expectations. Oncolytic adenoviruses are well tolerated and have shown to preferentially target and kill cancer cells. Importantly, they can convert immunosuppression locally into a proinflammatory state. Therefore, to improve the therapeutic efficacy of DC therapy, we armed oncolytic adenovirus with CD40 ligand (CD40L). CD40L is well known to regulate immune responses through its capacity to stimulate dendritic cells that lead to the activation of cytotoxic T-cells. In this study, we generated a novel virus Ad3-hTERT-CMV-hCD40L, which is fully serotype 3 adenovirus (Ad3). It features a human telomerase reverse transcriptase (hTERT) promoter for tumor specificity and expresses human CD40L (hCD40L) under a cytomegalovirus (CMV) promoter for induction of antitumor immune responses. Of note, human [1] and animal data [2] have shown the ability of Ad3 to successfully reach tumors through the intravenous route. In syngeneic studies in an immunocompetent model, DC therapy with our murine CD40L-armed adenovirus showed significant antitumor immune response. This enhanced therapeutic effect is associated with increased tumor specific T-cells and induction of T-helper type 1 immune response [2]. This was further evaluated in human peripheral blood mononuclear cells (PBMCs) humanized mice and treatment with hCD40L-armed adenovirus Ad3 and human DCs showed 100 percent survival in conjunction with tumor control [3]. To further deeply dissect if Ad3-hTERT-CMV-hCD40L can modulate tumor microenvironment, we established tumor histocultures derived from prostate and ovarian patients and evaluated the tumor viability, changes in immune cells especially DCs and the cytokine content of the tumor microenvironment over time post-adenovirus infection. Treatment with hCD40L- armed adenovirus showed that virally expressed hCD40L in the tumor microenvironment leads to significant activation of dendritic cells and induction of Th1 immune

responses. To conclude, CD40L armed oncolytic adenovirus 3 improves DC therapy by favorable alteration of tumor microenvironment. These findings support clinical trials where DC therapy is enhanced with oncolytic adenovirus.

Keywords : Dendritic cells, Oncolytic adenovirus 3, CD40L, T-cells.

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## B084 / Nociceptors control functions of dendritic cells

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Nociceptors – sensory neurons that respond to noxious stimuli by eliciting pain or itch – are increasingly beginning to be appreciated as controllers of the immune response. Notably, dendritic cells (DCs) – myeloid cells of the immune system – were previously found to engage in a physical interaction with nociceptors in the skin and be impacted by the actions of the latter. Strikingly, while some functional outcomes of the interaction have been described, our mechanistic understanding of the underlying molecular communication network is rudimentary at best and a bigger picture pertaining the modalities and outcomes of the interaction is missing entirely.

In order to address this gap in our understanding, we developed a novel in-vitro co-culture system for nociceptors and DCs, which allowed us to dissect the interaction under simplified settings. Importantly, and in agreement with previous reports, we observed that nociceptors enhanced the ability of DCs to produce pro-inflammatory cytokines in response to a TLR7 agonist, Imiquimod (IMQ), and we were able to extend this observation to other defined TLR agonists as well as whole pathogens. Strikingly, we noticed that such effect was only apparent if DCs and nociceptors were allowed a direct physical contact and, using live cell imaging, we observed that DCs and nociceptors engaged in a tight and dynamic physical interaction. Functionally, activation of nociceptors was sufficient to drive membrane depolarization and calcium influx into the cytoplasm of the interacting DCs. Lastly, in agreement with a model in which activation of nociceptors helps DCs that received suboptimal stimulus reach the threshold of activation, we demonstrated that rather than increasing the cytokine output of single cells, nociceptors increase the proportion of DCs that produce cytokines within a population.

In order to address more broadly how nociceptors impact on the function of DCs, we performed RNA sequencing experiments, in which we compared DCs cultured and stimulated in isolation or in the presence of nociceptors. In total, in excess of 1, 400 genes were differentially regulated by the presence of nociceptors and unsupervised hierarchical clustering analysis divided them into 2 large gene-sets: A gene-set that was impacted by the presence and activation of nociceptors, and a gene-set that was mainly affected by the activation of DCs and then modulated by nociceptors. Gene-set enrichment analysis (GSEA) of genes modulated by nociceptors during IMQ stimulation showed that the most upregulated pathways included glycolysis and hypoxia response pathways, consistent with a higher degree of activation of DCs in the presence of nociceptors. Additionally, a significant downregulation in the unfolded protein response and apoptotic signaling, in particular in the cDC1 subset was revealed. Next, analysis of the changes induced by the presence and activation of nociceptors revealed that a number of genes involved in the inflammatory responses were upregulated. Among them, notably, we found a shared gene signature between cDC1 and cDC2 subsets that contained genes involved in the sentinel function of DCs. In particular, we observed that IL1b was upregulated in the presence of nociceptors and, activation of nociceptors conferred even higher degree of upregulation. Crucially, we were able to confirm this induction of pro-IL1b protein in DCs both in vitro and in vivo, and we identified the CGRP family of neuropeptides as nociceptor-derived signals mediating this effect.

In summary, we have developed a novel in-vitro culture system for nociceptors and dendritic cells. We demonstrated its applicability by reproducing previous in-vivo observations for which we also, for the first time, provide a mechanistic explanation and, finally, we used our setup to describe previously unappreciated effects that nociceptors have on DCs, in particular their ability to enhance sentinel function of DCs.

Keywords : Neuroimmunology, Dendritic cells, Nociceptors, Innate Immunity.

## B085 / Sorafenib initiates macrophage-natural killer cell crosstalk by macrophage pyroptosis

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Sorafenib, a multi-tyrosine kinase inhibitor for the treatment of unresectable hepatocellular carcinoma (HCC), shows antiangiogenic and cytotoxic effects. However, impact and mechanisms of sorafenib on the anti-tumor immune response are not yet fully understood. The small fraction of HCC patients who achieve complete responses associated with dermatologic reactions imply an additional immunomodulatory mechanism for sorafenib.

To investigate the mechanism of action of sorafenib in more detail, the inducible albumin-specific SV40 T antigen (iAST) mouse model and the fragment-based Hep-55.1c mouse model were used [1]. In vivo cell depletion experiments in the iAST mice showed that the depletion of macrophages completely abrogates the antitumoral effects of sorafenib. Macrophages were identified as key mediators of the sorafenib-induced tumor growth inhibition. Furthermore, the concurrent depletion of macrophages and natural killer (NK) cells displayed an acceleration of HCC tumor growth and thereby indicates a crosstalk of macrophages and NK cells. In vitro caspase 1 analysis in sorafenib-treated macrophages revealed the induction of pyroptosis followed by IL1B and IL18 cytokine release. As a result, cytotoxic NK cells become activated when co-cultured with sorafenib-treated macrophages. In vivo cytokine blocking demonstrated that sorafenib efficacy is abolished after inhibition of IL1B and IL18. In addition, sorafenib was found to downregulate major histocompatibility complex class I expression of tumor cells, which may reduce the tumor responsiveness to immune checkpoint therapies and favor NK-cell response. We further show that this mechanism acts independently of the inhibition of angiogenesis. In iAST mice, sorafenib therapy led to vascular regression and showed strong reduction of tumor growth. However, single antiangiogenic therapy with anti-VEGF antibody did not lead to decreased to tumor progression.

Taken together, our findings unravel a novel immunomodulatory mechanism of sorafenib involving macrophage pyroptosis and unleashing a NK cell response. This suggests sorafenib as a promising immunotherapy combination partner and maximizes its utility for HCC treatment.

Keywords : Sorafenib, Pyroptosis, NK cells, Macrophages.

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## B086 / Phagocytic clearance of microparticles with cancer cell-like physical properties by macrophages

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The abundant presence of macrophages at tumoral sites combined with their tumoricidal potential allows exploitation of macrophages to suppress tumors by cancer-cell phagocytosis.

However, our lack of understanding of the unique challenges associated with phagocytosis of cancer cells hinders us from fully exploiting this tumoricidal potential of macrophages. In particular, macrophage clearance of targets by phagocytosis seems to be target-rigidity dependent, and less efficient for clearing "soft" targets like cancer cells than bacteria, which are typically ~1000 fold more rigid than human cells. It is currently poorly understood why low target rigidity leads to reduced uptake by macrophages, and if macrophages can adapt phagocytic mechanisms for more efficient uptake of soft targets. Here, we developed deformable hydrogel microparticles as a model system to study phagocytosis in unprecedented detail, and using targets that accurately mimic cellular physical properties. [1] These hydrogel particles have cell-like sizes (~10 um) and rigidities (Young's modulus 0.1 - 10 kPa) and can readily be conjugated with a variety of ligands to mimic the cellular surface. We show that IgG-functionalized microparticles are taken up by J774.1 macrophage-like cells in a rigidity dependent manner, being 6-fold less efficient for particles with similar rigidity to cancer cells than stiffer targets. Moreover, the particles are deformed during phagocytosis, which we can use as a direct readout of the molecular mechanisms and cytoskeletal organization that are required for phagocytosis. We observed highly localized target deformations by the phagocytes and distinct steps in the phagocytic process. Initially, we observe outward directed pushing from the phagocytic cup base. During subsequent phagocytic cup growth, we see that the majority of the deformation is localized in a ring that is initially irregular but becomes more uniform during cup closure. Surprisingly, strong localized punches at the cup base occur in these stages. After cup closure, we observe the strongest macrophage-induced target deformations, seemingly pushing the engulfed target into the cell. Interestingly, when functionalizing particles with phosphatidylserine, the dominant prophagocytic signal for removal of apoptotic cells which is also present in increased levels on cancer cells, we observe a clearly distinct uptake mechanism. Current work focuses on using magnetized particles in full-genome screening assays for identifying key regulators that affect phagocytic clearance of soft targets. Our novel approach gives us unprecedented detail of the mechanism of phagocytosis of soft targets by macrophages. Moreover, the presented methodology is can be applied broadly in immune cell biology.

Keywords: macrophages, biomechanics, phagocytosis.

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## B087 / Preclinical mouse models for immune-oncology R&D

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The recent clinical success of immune checkpoint modulators has stimulated immune-oncology research and the identification of new tumor immunology targets. Despite all progress, many preclinical development remain challenging as available models are of limited translational predictively. Preclinical models, which combine heterogeneous tumor and immune compartments, are not only needed for development of new therapies, but also for evaluation of immunotherapy combinations, elucidating the mechanisms of action of immunotherapies and identification of clinically relevant biomarkers beyond the approved PD-L1 predictive biomarker.

Syngeneic mouse models have been used in cancer research since decades and as they provide a functional immune cell/tumor cell system, we have re-evaluated them for IO. We screened their response to PD-L1 inhibitor treatment in combination with radiation, to investigate whether irradiation could increase immunogenicity of the tumors and sensitivity to treatment with checkpoint inhibitors. Further to this, we developed two technologies to generate mice with a humanized immune system by either transplantation of human peripheral blood mononuclear cells (PBMC) or human hematopoietic stem cells (HSC). These mice with engrafted human immune cells were applied to study the efficacy and mode of action of new immunotherapeutic drug and combinations.

From our syngeneic tumor models, 4 out of 9 did not respond to PD-L1 antibody treatment (= fully immune resistant). Some models showed a tumor growth delay by the checkpoint inhibitor treatment, however only the P388 leukaemia and the 4T1 breast cancer model were significantly inhibited (= partly immune responsive). Complete tumor regressions were not observed in the syngeneic models. Our panel of syngeneic tumor models was further tested towards response to a combination of local tumor irradiation and PD-L1 antibody treatment. Synergistic efficacy of the combination was observed in the models P388 and MC38 (70% and 95% tumor growth inhibition). This activity was in correlation with the accumulation of tumor infiltrating immune cells.

Mice humanized with PBMC provide an established and robust model for IO research. These mice can be co-inoculated with cell or patient derived tumor xenografts and have been used for the evaluation of bispecific antibodies or check point inhibitors (Reference Stecklum et al.). Recent studies have demonstrated improved tumor growth in models with HLA-match between PBMC donor and xenograft tumor. Further we have demonstrated increased efficacy due to immune-stimulatory effects for therapeutic antibodies in our models. Treatment with trastuzumab was more effective in breast cancer models on humanized mice. We have further shown synergistic effects for a combination of chemotherapy with PD-1 inhibition in breast cancer PDX on humanized mice. The most sophisticated humanized model for IO can be generated by a reconstitution of a human immune system in bone marrow depleted immunodeficient mice by engrafting human hematopoietic stem cells. At the time when the human immune system is developed, patient-derived tumors were transplanted on these reconstituted humanized mice. We have demonstrated efficacy of PD-1 or PD-L1 inhibitors in these humanized PDX models.

Keywords: mouse models, syngeneic, humanized.

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## B088 / The increased regulator T cell by IP-10 mediated TGF-β in EGFR mutant NSCLC after EGFR TKI therapy

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Background. Studies on the immune microenvironment of EGFR mutant lung cancer have been limited. We analyzed the effect of immune microenvironments on the development of EGFR-TKI resistance in EGFR-mutated lung cancer. Methods. The EGFR mutant lung cancer cell lines (HCC827 and HCC4006) were co-cultured with activated PBMC for 72 hours with EGFR-TKI. Changes of cytokines/chemokines in the media, PD-1 expression of CD8+ T cells, regulatory T cells fraction and transcriptome analysis of tumor cells were analyzed. We also performed immune profile analysis of fresh tissues of 21 surgically resected NSCLC (7 EGFR mutant and 14 EGFR wild) by multicolor FACS.

results. IFN-γ, IL-6, VEGF, TGF-β1 and IP-10 were significantly increased after co-culture but did not decrease after EGFR-TKI. PD-L1 expression on tumor cells increased after co-culture (p=0.08 in HCC827 and p=0.09 in HCC4006) but did not decrease after co-culture with activated PBMC and EGFR-TKI treatment (p=0.36 in HCC827 and p=0.45 in HCC4006). PD-1 expression of CD8+ T cell co-cultured with HCC827 or HCC4006 did not change, however proportion of regulatory T cell increased after co-culture with HCC827 or HCC4006 (p=0.05 and p=0.08, respectively) and did not decrease during EGFR-TKI treatment. Proportion of regulatory T cell in co-cultures with A549 or H1975 (erlotinib resistant cell line) did not change during co-culture or EGFR-TKI treatment. The inhibition of IP-10 by siRNA significantly decreased TGF-β1

expression and proportion of regulatory T cells in co-cultured mutant EGFR lung cancer cell with EGFR-TKI treatment. Transcriptome analysis by RNA sequencing showed 1747 gene sets were differentially expressed in EGFR-TKI treated EGFR mutant cell line co-cultured with activated PBMC compared to EGFR-TKI treatment alone. Interferon gamma response pathway (NES 2.65, FDR q<0.1) was most significantly changed. Immune profile analysis of human EGFR mutant lung cancer showed that markedly heterogeneity in total lymphocyte infiltration, as low as 8.03% or as high as 44.7% of live cells. Among immune cells, proportion of CD4+/CD3+T cells in EGFR mutant groups was increased compared to EGFR wild group(62.7 3 2.96 vs 55.14 3 5.1% among CD3+ T cells) and proportion of FOXP3+CD25+CD4+ Treg in EGFR mutant group tended to increase compared to EGFR wild group (1.35230.4 vs 0.74 3 0.16%, p=0.256).

Conclusion. The increased regulator T cell by IP-10 mediated TGF-β is considered to be important in EGFR mutant NSCLC in immune suppressive microenvironments and EGFR-TKI resistance.

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Keywords: Regulatory T cell, Lung cancer, EGFR TKI.

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## B089 / Identification of a synergistic multi-drug combination active in cancer cells via the prevention of spindle pole clustering

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Clear cell renal cell carcinoma (ccRCC) represents about 70% of kidney tumors and is the most aggressive type, with around 30% of patients presenting metastases at diagnosis. ccRCC has been reported to rapidly acquire resistance to conventional treatments. Drug combinations can improve cancer treatment efficacy, while reducing side effects and the emergence of drug resistance. Nevertheless, finding optimal drug combinations is challenging due to the high number of combinatorial possibilities. Using the validated streamlined-Feedback System Control technique, we performed a search in ccRCC cells (786-O) and non-malignant renal cells and identified a synergistic low-dose four-drug combination (C2) with high efficacy and negligible toxicity. Cell-cycle analysis showed that C2 perturbs cell divison and arrests the cells in G2/M. Performing live-cell imaging, we discovered that C2 inhibits multipolar spindle pole clustering, a survival mechanism employed by cancer cells with spindle abnormalities. This phenotype was also observed in 786-O cells resistant to sunitinib, the first-line ccRCC treatment, and validated in melanoma cells with distinct percentages of supernumerary centrosomes. We conclude that the C2-treatment efficacy is linked to spindle abnormalities in cancer cells. Compounds that prevent pole clustering have emerged as a promising therapeutic agent, since they specifically target cancer cells that form multi-polar spindles, e.g. abnormal centrosome numbers, but do not affect mitotic progression in non-malignant cells with normal centrosome numbers.

Keywords: drug combinations, drug synergy, multipolar spindle pole clustering, clear cell renal cell carcinoma.

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## B090 / Permissivity and mode of action of oncolytic viruses in human tumors ex vivo

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Oncolytic virotherapy represents a promising therapeutic area especially with regard to combination treatment with cancer immunotherapeutics. Oncolytic viruses (OVs) are biologically active and exert different mode of actions to eradicate the tumor bulk: OVs not only directly infect and kill tumor cells (oncolysis), but could also boost immune-stimulatory responses and activated immune cells that could recognize, infiltrate and attack the tumor. To this end, efficacy and mode of action of the OV VSV-GP were demonstrated in a variety of human tumor cells lines and murine tumor models, but a functional relevance for the human tumor ecosystem including the tumor microenvironment remains elusive2. Thus, there is the urgent need to set up pre-clinical model systems resembling this complex human tumor contexture, which could complement the pre-clinical tool box. Here, we have established human tumor ex vivo cultures derived from head and neck squamous cell carcinoma (HNSCC)-patients, preserving the polyclonal tumor tissue as well as the stromal and the immune compartment. From our initial experiments we have learned that the OV VSV-GP can infect and eradicate human HNSCCs ex vivo. Currently, we aim to understand the mode of action of the OV, particularly with regard to the complex interplay of the OV and the tumor microenvironment. Unveiling differences between susceptible and non-susceptible tumors could help to improve OV therapy and identify potential combination partners.

Keywords: oncolytic virus, cancer immunotherapy.

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# T cell exhaustion – mechanisms of resistance

## B091 / Tumor-infiltrating CD4+TIM-3+ T cells have suppressive, exhausted and metastatic gene profile in colorectal cancer patients

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T cell immunoglobulin mucin-3 (TIM-3) is an immune checkpoint identified as one of the key players in regulating tumor immune biology. Studies have shown that TIM-3 is upregulated in the tumor microenvironment (TME) of colorectal cancer (CRC). However, the precise role of TIM-3 in the CRC TME is yet to be elucidated. Here, we performed phenotypic and molecular characterization of TIM-3+ T cells in the TME and circulation of CRC patients by prospective analyses of tumor tissues (TT, TILs), normal tissues (NT, NILs) and peripheral blood mononuclear cells (PBMC). Phenotypic characterization of PBMC from 34 and NILs and TILs from 27 CRC patients was assessed by flow cytometric analyses. RNA-Sequencing of sorted, pure CD4+TIM-3+/- and CD4+CD25+ TILs was performed from 3 and 2 patients, respectively. TIM-3 was upregulated on both CD4+ and CD8+ TILs. CD4+TIM-3+ TILs express higher levels of T regulatory cell (Tregs)-signature genes, including FoxP3 and Helios, compared with their TIM-3- counterparts. Transcriptomic and ingenuity pathway analyses showed that TIM-3 expression activates inflammatory and metastatic pathways. Moreover, NF-κB-mediated transcription factors were upregulated in CD4+TIM-3+ TILs, which could favor proliferation/invasion and induce inflammatory and T-cell exhaustion pathways. Additionally, we found that TIM-3+ TILs have more invasive and metastatic characteristics, compared with conventional CD4+CD25+ Tregs in the CRC TME. Our data suggest that TIM-3 expression in T cells in the TME can mediate T-cell exhaustion and promote metastasis. TIM-3 may therefore be utilized as a prognostic biomarker or therapeutic target in CRC patients.

Keywords: TIM-3, Colorectal Cancer, Tumor microevironment.

## B092 / Barking up the wrong tree: Anti-CD137 immunotherapy induces T cell exhaustion in CD8+ T cells

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CD8+ T lymphocytes represent actually the most promising therapeutic target against cancer. Although CD8+ T cell activation is mainly triggered by TCR signaling, many activating and inhibitory receptors tune the functions of these cells. Promising results obtained by monoclonal antibodies (mAbs) targeting CTLA-4 and PD-1 inhibitory receptors in metastatic cancer demonstrate the importance of defining key receptors that control cytotoxic lymphocyte anti-tumor reactivity. The surface glycoprotein CD137 (TNFRSF9, 4-1BB) is a costimulatory member of the tumor necrosis factor receptor superfamily expressed by activated T cells. We recently demonstrated using the most relevant mouse model for multiple myeloma (MM) that anti-CD137 monoclonal antibodies (mAbs) have potent anti-myeloma effects (Guillerey, Ferrari de Andrade et al. 2015). Indeed, anti-CD137 mAbs rapidly led to the accumulation of activated effector memory CD8+ T cells in the tumor microenvironment that accounted for CD137 anti-tumor effects (Guillerey, Nakamura et al. 2019). Surprisingly, global transcriptomic analysis also revealed that CD137 stimulation progressively induced the expression genes related to T cell exhaustion in the tumor microenvironment. Consistent with these results, we found that anti-CD137 mAb treatment led to the accumulation of effector CD8+ T cells with reduced effector functions that were characterized by the expression of multiple immune checkpoints (IC) including PD-1, Tim3 and Lag-3. Single-cell RNA sequencing analysis confirmed that CD137 engagement gradually alters CD8+ T cells transcriptional landscape ultimately expanding CD8+ T cell clusters overexpressing archetypal "T cell exhaustion" genes. While blocking immune checkpoints upon anti-CD137 mAb treatment didn't reinvigorate CD8+ T cells nor improved anti-CD137 anti-tumor efficacy, we found that CD137 stimulation induced important metabolic changes that may account for anti-CD137 driven T cell dysfunctions. Finally, we found that T cell specific deletion of Eomes prevented anti-CD137 driven T cell exhaustion suggesting a key role for this transcription factor in anti-CD137 mAbs induced dysfunctional program. Altogether these results uncover an unsuspected role for CD137 activation receptor in driving a profound T cell exhaustion state that might not be reversed by immune checkpoint blockade. Further investigations are required to dissect at the molecular level the mechanisms and the signaling underlying CD137 activation versus exhaustion program.

Keywords: cytotoxic CD8+ T cell, T cell exhaustion, immunotherapy, CD137 (4-1BB).

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## B093 / MDR1 expression identifies a Th1.17 effector population resistant to chemotherapy treatments that could play a pivotal role in anti-cancer immunity

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The Multi-Drug Resistance transporter 1 (MDR1) is an ABC transporter able to exclude a wide set of xenobiotics and anti-cancer drugs from cells through an ATP-dependent process. Widely studied on multiple tumor cell types, MDR1 is involved in chemo-resistance because it limits intracellular drug accumulation such as Paclitaxel and Doxorubicin used in treatment of breast (BC) and ovarian (OC) cancers. In the T cell compartment, MDR1 is largely expressed on CD8+ T cells whereas on CD4+ T cells, its expression is enriched on a specific effector population (Teff) displaying a Th1.17 profile and expressing the ectonucleotidase CD73 (Gourdin et al Cancer Res 2018). We demonstrate that MDR1 is functional on this population and delineates a potent polyfunctional subset co-producing IL-17A and IFNγ. In vitro treatments with increasing doses of Paclitaxel in presence of a TCR signal show that MDR1+ Teff resist better to chemotherapy and proliferate more than their MDR1neg counterpart, resulting in an enrichment in polyfunctional Th1.17 subset. In addition, analyses of BC patients' blood treated in neo-adjuvant setting with Paclitaxel and/or Doxorubicin reveal an increased proportion of MDR1+ Teff as well as an increase in Th1.17 cells compared to untreated patients. An increased polyfunctionality of this population is also observed on treated patient blood compared to untreated ones. To complete these ex vivo data, we are analyzing a retrospective cohort of breast cancer patients treated with neoadjuvant chemotherapy. Modulation of MDR1+ Teff in blood and their localization in tumor tissue before and after treatment are under evaluation. Altogether, these data indicate the existence of a MDR1+ Th1.17 population which resists to chemotherapy treatments and represents a potent actor in anti-cancer immunity. However, due to its expression of CD73 that generates immunosuppressive adenosine from extracellular AMP, the antitumor function of this MDR1+ Teff population might be abolished in tumor microenvironment. These observations argue for the development of clinical trial combining MDR1-sensitive chemotherapy with neutralizing anti-CD73 antibodies.

Keywords: MDR1, chemotherapy resistance, Th1.17, CD73.

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## B094 / Alternative splicing of the receptor SLAMF6 reveals a novel regulatory mechanism of T cell activation and can be used for cancer immunotherapy

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SLAM (signaling lymphocytic activation molecule) is a family of receptors widely expressed on hematopoietic cells. SLAMF6 is a homotypic receptor abundantly expressed on CD8+ T lymphocytes and thus of interest for its role in anti-tumor response. Recently we evaluated two splice isoforms of the SLAMF6 gene: The 'canonical' sequence, and SLAMF6Δ17-65 missing part of exon-2. In this work we set to evaluate the immune modulatory role of the long and the short isoforms of SLAMF6 and test their effect on anti-tumor immunity.

SLAMF6 splice isoforms were identified by RT-PCR and in RNA-seq databases from human donors and lymphoid cell lines, in different activation states. Melanoma lines aberrantly expressing each isoform were produced to generate a model of trans-activation of T cells via SLAMF6. In parallel, selective expression of SLAMF6Δ17-65 in lymphocytes was achieved in Jurkat cells using CRISPR-Cas9 genome editing.

To identify transcript levels of SLAMF6 isoforms in both healthy individuals and in cancer patients receiving PD1-inhibitors, samples were collected from patients before and during anti-PD1 therapy, cells were sorted to CD8+ subsets and qPCR was performed. Finally, to shift the splicing, antisense-oligonucleotides (ASO) were transfected into Jurkat cells using electroporation.

Melanoma cells aberrantly expressing canonical SALMF6 had distinct inhibitory effect on cognate TILs. However, to our surprise the opposite was observed with SLAMF6Δ17-65. Melanoma expressing this isoform enhanced IFN-γ production by cognate TILs significantly and reproducibly. In line with this, the exclusive expression of the short isoform in Jurkat cells was associated with a three-fold increase in IL-2 secretion. Therefore – the canonical SLAMF6 is an inhibitor for T cell activation, while the shorter splice isoform - SLAMF6Δ17-65 is a strong activator.

Using existing databases and our-own human-derived T cell samples, we showed that all SLAMF6 isoforms are constitutively apparent on T-cells. However, different isoform ratios were found in CD8+ subsets, determined by their differentiation states. In melanoma patients receiving PD-1 inhibitory antibody, a transition was noted in isoforms ratio, favoring a rise in the shorter isoform, which was most emphasized in patients with auto-immune side effects (n=7). Lastly, using ASO designed to target SLAMF6 splice junctions, we managed to shift the splicing in Jurkat cells, increasing the short isoform on the account of the canonical isoform. As an outcome, cells expressing higher levels of SLAMF6Δ17-6 had a significantly improved function.

In this work we showed that SLAMF6Δ17-65 isoform has a strong agonistic effect on T cell activation while its canonical isoform is an inhibitor. The change in isoform ratio observed during anti-PD-1 therapy may suggest a new regulatory mechanism that T cells adopt along their activation. The agonistic effect achieved by splice-diverting ASO may be exploited in the future for cancer immunotherapy, as part of adoptive cell therapy for example.

Keywords: Exhaustion, Alternative splicing, Immunotherapy, Antisense-oligo.

## B095 / Harnessing CD8+ T cell antitumor responses by manipulating extracellular ATP signaling

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Extracellular adenosine triphosphate (eATP) is a well-described tissue-damage associated signal and is recognized by purinergic receptors in mammals. Among those receptors, P2RX7 is preferentially expressed in immune cells, activating both innate and adaptive immune responses. We recently discovered that P2RX7 is crucial for the generation and maintenance of long-lived circulating and resident memory CD8 T cells, through control of the mitochondrial function and viability of CD8 T cells (1). CD8 T cells are pivotal for tumor control, and therapies that aim to harness CD8 T cell function and overcome exhaustion are featured anti-cancer strategies. However, the success rate of such therapies is still not optimal, partially due to our lack of knowledge about the role of the tumor microenvironment in shaping CD8 T cell biology. Interestingly, using a chronic viral infection system, we found that P2RX7 is required for control of viral titers and expansion of PD1-blockade reactive CD8 T cells (1). Consequently, P2RX7-deficient CD8 T cells do not respond to checkpoint blockade. Considering these results, and the aforementioned role of CD8 T cells in tumor control, we next sought to investigate if eATP sensing through P2RX7 could influence CD8 T cell-mediated antitumor responses. By using adoptive transfer therapy into a mouse melanoma model, we observed that cell-intrinsic P2RX7 is required for CD8 T cells to optimally control tumors in the presence of IL-12 priming. Mechanistically, P2RX7 signaling was crucial for CD8 T cells to initially infiltrate tumors, as well as to survive and acquire resistance to cell exhaustion – a hallmark feature of IL-12-stimulated CD8 T cells (2). P2RX7-deficient CD8 T cells activate and respond to IL-12 in vitro normally, as measured by proliferation and up-regulation of in vitro cytokine production. The mitochondrial respiration rates of these cells are decreased, however. Correlating with this, P2RX7-deficient CD8 T cells had decreased mitochondrial mass and membrane potential in vivo, after tumor infiltration. This suggests P2RX7 signaling is important for CD8 T cells to acquire a tumor microenvironment-resistant phenotype, which correlates with sustained mitochondrial function. Finally, we assessed whether P2RX7 agonism could be used to boost CD8 T cell-mediated tumor control. In vitro activation in the presence of BzATP (a P2RX7 agonist) mediated increased ability of CD8 T cells to infiltrate and control tumors, in comParison to Vehicle-treated CD8 T cells. Overall, these results show P2RX7 is crucial for CD8 T cells to respond to distinct situations where chronic antigen is present, including tumors. Moreover, they offer exciting prospects for CD8-targeted P2RX7 agonism to be a strategy for boosting antitumor immunity in future translational studies.

Keywords: P2RX7, CD8 T cell, Exhaustion, Melanoma.

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## B096 / TOX and TOX2 transcription factors cooperate with NR4A transcription factors to impose CD8+ T cell exhaustion

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T cells expressing chimeric antigen receptors (CAR T cells) have shown impressive therapeutic efficacy against leukemias and lymphomas1, 2, 3; however, they have not been as effective against solid tumors because chronic stimulation with tumor antigens causes them to enter a hyporesponsive ("exhausted" or "dysfunctional") state. Here we show that the high-mobility group (HMG)-box transcription factors TOX and TOX24, 5 are highly expressed in CAR-expressing exhausted (PD-1highTim3high) CD8+ tumor-infiltrating lymphocytes (TILs). CAR TILs deficient in both TOX and TOX2 (TOX DKO) suppressed tumor growth and

prolonged survival of tumor-bearing mice, compared to wild-type (WT), TOX-deficient or TOX2-deficient CAR TILs; they also showed increased effector function with decreased TCF1 expression and decreased expression of the inhibitory receptors PD-1, LAG3 and CD160. Like NR4A transcription factors6, TOX and TOX2 are critical for the transcriptional program of hyporesponsiveness downstream of NFAT7, 8. Disruption of TOX expression or activity could be a promising strategy for cancer immunotherapy.

Keywords: TOX, TOX2, NR4A, NFAT.

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## B097 / CD4 help is required for the generation of a transcriptionally distinct cytolytic CD8 T cell subset that protects against chronic viral infection and cancer

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"CD4 help" is crucial to sustain the function of exhausted CD8 T cells, yet the detailed mechanisms by which CD4 help regulates CD8 T cell differentiation and function during chronic viral infection remain elusive. Here, using single cell RNA-sequencing (scRNA-seq) we show that CD8 T cells responding to persistent LCMV Cl13 infection are more heterogeneous than previously appreciated, with three transcriptionally distinct subsets dominating the late phase of the antiviral response: Slamf6+ progenitor, Pdcd1+ exhausted, and Cx3cr1+ cytotoxic T cell subsets. Notably, although exhausted T cells and Slamf6+(TCF-1hi) progenitor cells have recently been described, our findings uncover the formation of a previously unrecognized CD8 T cell subset that exhibits potent cytolytic function. This subset is characterized by high expression of killer cell lectin-like receptors Klre1 and Klra9, the chemokine receptor CX3CR1, and the transcription factors T-bet and Zeb2. Importantly our sc-RNA-seq and flow cytometric analyses demonstrate that differential expression of cell surface receptors CX3CR1 and Ly108 (encoded by Slamf6) can distinguish these 3 major CD8 T cells populations, with PD-1hi exhausted cells lacking expression of either Ly108 or CX3CR1. Ex vivo functional analyses of these 3 major subsets indicated that Ly108+ CD8 T cells exhibit an enhanced capacity to co-produce IFN-g and TNF-a upon GP33-41 peptide stimulation, whereas the CX3CR1-Ly108- subset displayed the lowest potential to produce pro-inflammatory cytokines, consistent with their exhausted phenotype. Notably, we further identified that CX3CR1+ CD8 T cells displayed increased expression levels of granzyme B, which correlated with their augmented cytotoxicity against peptide-pulsed target cells. To determine whether CX3CR1+ CD8 T cells are required for control over chronic viral infection, we generated CX3CR1 DTR dLCK Cre mice, which allows for the selective depletion of CX3CR1+ T cells in vivo upon administration of diptheria toxin. Strikingly depletion of CX-3CR1+ CD8 T cells resulted in over 5-fold increases in circulating viral copy numbers, thereby demonstrating a protective role for the CX3CR1+ T cell subset in limiting viral replication. To gain insight into the lineage relationship between Slamf6+, Pdcd1+ and Cx3cr1+ CD8 T cells, we employed Sc trajectory modeling using the program Monocle. Our analyses predicted that Ly108+ CD8 T cells can give rise to both CX3CR1-Ly108- and CX3CR1+ subsets, a finding that was later confirmed via adoptive transfer experiments. To determine how CD4 help impacts this intricate process of CD8 T cell differentiation, we employed use of either a transient CD4 T cell-depletion model, or co-adoptive transfer experiments with WT and Il21r-/- P14 cells (which express a transgenic TCR specific for GP33-41). Notably, depletion of CD4 T cells or deletion of IL-21R signaling in P14 cells abrogated the development of the CX3CR1+ CD8 T cell subset, indicating a critical role for CD4 help in facilitating the differentiation of cytotoxic CX3CR1+ T cells. Moreover, PD-L1 blockade, an immunotherapy known to partially reverse T cell exhaustion during persistent infection and cancer, failed to rescue this differentiation defect of "un-helped" CD8 T cells. Lastly, using a B16-F10 melanoma model, we found that the majority of TILs coordinately lacked expression of both CX3CR1 and Ly108, consistent with a high level of T cell exhaustion occurring in the tumor microenvironment. Remarkably, provision of IL-21+CD4 help in the form of ACT was sufficient to induce over a 2-fold increase in the development of CX3CR1+ CD8 TILs, which was associated with enhanced control over tumor growth. Taken together, our findings demonstrate that CD4 help plays a critical role in the generation of protective CX3CR1+ CD8 T cells, and that this differentiation checkpoint may potentially be exploited for the purpose of ACT.

Keywords: CD4 help, single cell RNA-sequencing, CD8 T cell differentiation, LCMV Cl13.

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## B098 / CD226 absence affects TCR responsiveness and restrains tumor infiltrating T lymphocyte functions.

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Although immune checkpoint blockade (ICB) such as using anti-PD-1 has represented a turning point in cancer care, clinical responses are not observed in the majority of cancer patients. The mechanisms underlying this lack of responsiveness are still poorly understood and finding additional signals that regulate CD8+ T cell anti-tumor functions has become a major priority.

While most of the experimental strategies actually focus on the identification of additional inhibitory receptors restraining anti-tumor reactivity of CD8+ T cells, the importance of activating receptors with regards to anti-tumor CD8+ T cell functions and ICB efficacy remains to be better understood. CD226 (DNAM-1) is an adhesion molecule initially described for its ability to stimulate NK cell and CD8+ T cell-mediated cytotoxicity (1). Its ligands, the nectin and nectin-like receptors CD112 and CD155, are often expressed on cancer cells and CD226 deficiency was shown to reduce tumor immunosurveillance in a wide number of tumor mouse models (2). TIGIT and CD96, two inhibitory receptors that compete with CD226 for the same ligands, were recently identified as promising immunotherapeutic targets to restore immune reactivity against tumors highlighting the importance of the CD226 axis in the regulation of anti-tumor immune responses (3-4).

Interestingly, we recently found that loss of the activating receptor CD226 (DNAM-1) is a critical mechanism that alters CD8+ T cell responsiveness to TCR stimulation. Pre-clinical tumor mouse model confirmed that tumor development drives the differentiation of dysfunctional CD8+ T cell lacking CD226 expression through an Eomes-dependent mechanism. More importantly, our results demonstrated that CD8+ T cells fail to respond to ICB in the absence of CD226. Taken together our study suggests that CD226 loss represents an underappreciated determinant of CD8+ T cell dysfunction in the tumor microenvironment that may impact cancer patient response to immunotherapy.

Keywords: Co-stimulation, T lymphocyte, immunotherapy, nectin receptor family.

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## B099 / The Role of IL-27 signaling on Responses to Melanoma

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Checkpoint blockade and adoptive cell therapies harness the ability of T cells, critical mediators of the body's immune response to infection, to eliminate tumors. Entry of T cells into the tumor microenvironment exposes them to an array of regulatory signals that impact T cell differentiation and function. The immunomodulatory cytokine, interleukin 27 (IL-27), produced by tumor cells and immune cells, has been shown to exhibit pro- and anti-tumor functions via conflicting cellular mechanisms (1-4). Here we find that IL-27 receptor alpha (WSX1)-deficient mice exhibited reduced tumor burdens, by number of nodules and tumor area, following intravenous administration of B16 melanoma in comParison to wildtype mice. Furthermore, we find that tumor nodules in the lungs of WSX1-deficient mice have increased infiltration of CD3+ cells observed by histological analysis in comParison to tumor nodules in wildtype mice. The production of IL-27 in the lung by immune cells following tumor challenge and the improved resistance to intravenous dissemination of B16 melanoma by WSX1-deficient mice suggests that IL-27 signaling limits T cell infiltration and responses to melanoma within the lung. However, analysis of antigen-specific OT-I CD8+ T cells adoptively transferred into mice deficient for IL-27 cytokine followed by intravenous challenge with B16 melanoma expressing ovalbumin revealed a crucial role for IL-27 signaling in the expansion and differentiation of the tumor-specific T cell response. OT-I T cells in IL-27 deficient mice showed impaired proliferation and expansion that was accompanied by a reduced capacity to produce IFNg and expression of markers of tissue residency. These data were accompanied by impaired clearance of tumor in comParison to wildtype hosts that also received OT-I T cells. These data support a model where IL-27 plays two distinct roles, both inhibiting local immune responses to lung tumors and supporting the proliferation and differentiation of tumor specific CD8+ T cells. These findings highlight that improving our understanding of when and where pleiotropic cytokines such as IL-27 impact immune responses will be essential for the continued development of immunotherapies.

Keywords: IL-27, regulatory T cells, effector T cells, inhibitory receptors.

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## B100 / Characterizing the immune response in melanoma patients treated with neo-adjuvant Pembrolizumab

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Immune checkpoint blockade, including anti-PD-1 therapies, have had unprecedented success in treating various forms of cancer, including melanoma. Unfortunately, most patients still do not achieve durable clinical remission. The limited understanding of how these therapies work at the cellular and molecular level prevents the optimization required to improve patient outcomes.

We performed RNA-seq and ATAC-seq on four sorted PBMC subpopulations (naïve CD8 T cells, non-naïve CD8, non-naïve CD4 T cells, T regulatory cells) from patients with metastatic melanoma before and after Pembrolizumab treatment (1). We identified changes after treatment that were consistent with enhanced T cell activation and exhaustion. We also observed a distinct molecular signature in patients that experienced a tumor recurrence within one year of surgical resection. Interestingly, this signature was also present in the blood pre-treatment. These results indicate that it might be possible to predict clinical response to Pembrolizumab before treatment begins.

Keywords: Pembrolimab, Exhaustion.

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## B101 / Neoantigen-driven remodelling of the T cell landscape in NSCLC

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T cell responses to pathogen-derived epitopes provide indispensable host protection. In contrast, chronic antigen exposure drives accumulation of dysfunctional T cell pools at the expense of canonical memory and effector subsets, leading to a loss of T cell homeostasis, pathogen escape and fatal defects in immune control. In cancer, mutation-encoded neoantigens elicit T cell responses that sensitise patients to immune checkpoint blockade (ICB), yet the extent to which neoepitopes also skew local T cell differentiation and induce T cell dysfunction is unclear. Here, we integrate WES and RNAseq from surgically resected, untreated, early stage NSCLC specimens with paired high dimensional flow cytometry, MHC multimer screening and bulk- or sc-RNAseq of TILs to study neoantigen-driven T cell differentiation in the context of NSCLC evolution. Tumour mutational burden (TMB) correlated with intra-tumoural T cell activation (e.g. 4-1BB, HLA-DR expression on CD8+ T cells) and improved disease-free survival, supporting the prevailing hypothesis that neoantigens augment tumour immunogenicity. However, in tumours under high T cell selection pressure, characterised by evolution of immune escape mechanisms (e.g. HLA LOH, deleterious mutations or copy number loss in antigen processing/presentation machinery including B2M) TMB was linked to diminished tissue resident memory populations and expansion of dysfunctional T cells; highlighting a program of neoantigen-associated T cell dysfunction (Neo-Dys) that imbalances local T cell homeostasis. Neo-Dys was associated with worse outcome in multivariate models, independent of TMB, and occurred via a conserved molecular circuit that was validated in neoantigen-specific T cells and independent NSCLC cohorts. The Neo-Dys transcriptional network involved established inhibitory loci (ENTPD1, PDCD1, HAVCR2, LAG3) and several novel immunoregulatory genes under investigation as potential actionable IO targets. Taken together, these data are consistent with a dual role for neoantigens in treatment-naïve cancer in which augmentation of tumour immunogenicity may be progressively counterbalanced by the onset of Neo-Dys and evolution of immune escape mechanisms.

Keywords: T cell dysfunction, Neoantigen, Lung cancer, Immune profiling.

## B102 / Exhausted Tissue Resident Memory CD8 T Cells Encompassing Tumor-Specific Cytotoxic T Lymphocytes are Predictive of Clinical Response to PD-1 Blockade in Epithelial Malignancies

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While our understanding of T cell exhaustion is widely based on mouse models, in depth analysis of T cell exhaustion in cancer patients will provide cues of tumors sensitivity to immune checkpoint blockade (ICB) (1). Here, in ovarian, cervical and head and neck cancers, 3 epithelial malignancies exhibiting resistance to ICB, we combined phenotypic, single-cell RNA sequencing (scRNA-seq) and functional approaches to characterize exhaustion of tumor antigen-specific CD8 T cells at the tumor site. We show that along chronic stimulation of tumor-specific T cells, but not bystander cells, immune checkpoints (IC) expression is sequentially acquired leading to a population expressing the 4 IC under investigation, i.e. programmed cell death 1 (PD-1), T cell immunoreceptor with Ig and ITIM domains (TIGIT), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and T-cell immunoglobulin and mucin-domain containing-3 (TIM-3), that we named quadruple positive (QP) cells. Checkpoints incremental acquisition was accompanied by a sequential increase in the expression of tissue-resident memory T cell (Trm) markers (2, 3), of the ectonucleotidase CD39 (4), and of the transcription factor TOX associated to a T-cell exhaustion program in chronic infection models (5). Remarkably, QP cells exhibited significant loss of CD28, which could be reproduced by T-cell stimulation in the presence of transforming growth factor-beta (TGF-beta), a central cytokine of immune evasion. Despite their exhausted phenotype, QP cells were endowed with high cytotoxic potential and expressed the C-X-C motif chemokine receptor 6 (CXCR6), which could contribute, together with Trm markers, to their tumor residency and co-localization with tumor cells. Ex vivo phenotyping of circulating and tumor-infiltrating cancer antigen-specific T cells argued in favor of the in situ acquisition of the exhausted Trm-like phenotype by memory tumor-specific CD8 T cells once they infiltrate tumors. In addition, we show that circulating specific PD-1intCD28+ T cells respond to anti-PD-1 mAb by enhancing their proliferation in response to antigen stimulation. Instead, the same cells within tumor-infiltrating lymphocytes (TIL), which were PD-1hiCD28+/-, exhibited a reversal of their functional exhaustion. Finally and in agreement with their tumor specificity and responsiveness to PD-1 inhibition, QP cells, quantified by multiplex immunohistochemistry (6), were predictive of response to therapy and of overall survival in a cohort of 30 head and neck cancer patients treated by PD-1/ programmed death-ligand 1 (PD-L1) blockade therapy. Predictors of response to ICB will be instrumental for an optimized clinical output of current and future immunotherapies.

Keywords: T cell exhaustion, immune checkpoint blockade, CD8 T cells, tumor antigens.

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## B104 / Functionally specialized subsets of exhausted CD8+ T cells mediate tumor control and differentially respond to checkpoint blockade

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T cell dysfunction is a hallmark of many cancers, but the basis for T cell dysfunction and the mechanisms by which antibody blockade of the inhibitory receptor PD-1 (anti-PD-1) reinvigorates T cells are not fully understood. By comparing T cell exhaustion in chronic viral infection and cancer, we show that dysfunctional CD8+ TILs possess canonical epigenetic and transcriptional features of exhaustion that mirror those seen in chronic viral infection. Furthermore, we define the fundamental epigenetic landscape adopted by exhausted T cells in response to chronic exposure to antigen and inflammation, regardless of the disease state. Importantly, treatment with anti-PD-1 does not alter this core program in CD8+ TILs, suggesting novel therapeutic strategies will be needed to reverse exhaustion. Instead we show that the efficacy of anti-PD-1 therapy depends on a specific subpopulation of exhausted CD8+ tumor-infiltrating lymphocytes (TILs). Exhausted CD8+ TILs include a subpopulation of 'progenitor exhausted' cells that retain polyfunctionality, persist long term and differentiate into 'terminally exhausted' TILs. Consequently, progenitor exhausted CD8+ TILs are better able to control tumor growth than are terminally exhausted T cells. Progenitor exhausted TILs can respond to anti-PD-1 therapy, but terminally exhausted TILs cannot. Patients with melanoma who have a higher percentage of progenitor exhausted cells experience a longer duration of response to checkpoint-blockade therapy. Thus, approaches to expand the population of progenitor exhausted CD8+ T cells might be an important component of improving the response to checkpoint blockade. Moreover, given the intense interest in understanding the biomarkers that predict response to checkpoint blockade, our findings suggest that evaluating the specific state of exhaustion in CD8+ TILs, not just their numbers, may be an important predictor of immunotherapy response.

Keywords: Epigenetics, T cell exhaustion, Immunotherapy, Systems immunology.

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Miller*, Brian C., Sen*, Debattama R., et al. 2019. "Subsets of Exhausted CD8+ T Cells Differentially Mediate Tumor Control and Respond to Checkpoint Blockade." Nature Immunology 20 (3): 326–36. *these authors contributed equally

## B105 / Customizable T Cell Suppression/Dysfunction In Vitro Assay to Screen Candidate Small Molecule Drugs

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T cell exhaustion (TEX) is a phenomenon that describes a T cell differentiation continuum of hierarchical progressive diminution of effector function. Many factors have been attributed to this including multiple co-inhibitory receptor surface expression, altered transcription factor expression, epigenetic rewiring and dysregulated metabolism. Antigen persistence is necessary for driving TEX maintenance in both the chronic viral infection setting and cancer. Although TEX cells share many similarities within the two aforementioned settings, there are differences. These differences include qualitative and quantitative variation in co-inhibitory receptor expression as well as differences in transcription factor expression. In addition to persistent antigen exposure in cancer, tumor-infiltrating lymphocytes (TILs) within the tumor microenvironment (TME) encounter numerous tumor-mediated immunosuppressive metabolic byproducts, suppressive cytokines, hypoxia and cellular debris which converge to suppress T cell function and uniquely alter is transcription factor profile. These suppressed or dysfunctional T cells are incapable of mounting an optimal anti-tumor response in part due to lack of fitness in competing for glucose and oxygen. Our team has utilized the recall antigen potency assay as a means to screen immune checkpoint inhibitor (ICI) drug candidates. In this assay healthy human PBMCs are stimulated with peptide(s) and grown in culture for one week. Day 4 supernatants are functionally assayed by ELISA for IFNg secretion and cells are assayed on day 7 by flow cytometry for CD8+ or CD4+ T cell expansion by using a single or cocktail of pMHC tetramers. We have shown that in roughly 30% of donor PBMCs, ICI drugs such as pembrolizumab are able to boost both IFNg secretion and antigen-specific recall. One potential explanation for this effect is that the observed increase in T cell co-inhibitory receptor expression and presumed co-inhibitory receptor downstream signaling is reduced with ICI drugs releasing these T cells from the repressive effects of these co-inhibitory receptors. Further, this assay has the potential to screen for donors who are in vitro "responders" and "non-responders" to ICI drugs and is also being used to screen co-stimulatory agonists, peptide biologics and other drug classes.

We reasoned that we could adapt the antigen recall assay to more directly study TME-mediated suppression on T cells. For this we expand donor PBMC T cells with appropriate antigen(s) in the presence or absence of a TME inducer of T cell suppression (IoTS). So far, we have tested several TME tumor metabolites/soluble factors that have been shown in the literature to depress T cell anti-tumor activity and/or proliferation. We have shown that donor T cells stimulated with peptide(s) or even anti-CD3/CD28 beads are suppressed in their recall and IFNg secretion in the presence of these IoTS. Many dimensions of this assay have been optimized

including IoTS concentration and dosing regimen. In one example we show a donor whose antigen-specific T cell expansion is suppressed by high dosage IoTS. Screening of small molecule drugs capable of counteracting the IoTS effect revealed two of the three drugs tested were able to partially or almost fully restore antigen-specific T cell expansion depending on drug type or drug concentration. Drug candidates did not affect cell viability or significantly alter expression of co-inhibitory receptors beyond that induced by peptide alone, consistent with recent work describing an uncoupling between co-inhibitory receptor expression and T cell dysfunction. Establishment of this assay enables screening of ICI drugs as well as small molecule drug candidates that may be able to reverse TME-mediated T cell suppression.

Keywords: Cancer Immunology, T cell Exhaustion, CD8 T cell, co-inhibitory.

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## B106 / Accumulation of Long-chain fatty acids drives dysfunction in intra-pancreatic CD8+ T cells

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CD8+ T cells are master effectors of anti-tumor immunity and their presence at tumor sites has been correlated with favorable outcomes. Tumor-infiltrating CD8+ T cells often acquire an altered state of differentiation referred to as “exhaustion”, failing to control tumor outgrowth. The metabolic state and nutrient availability in the tumor microenvironment are among the main determinants of the functional fate of CD8+ T cells . However, how metabolic constraints imposed by the tumor microenvironment can dampen their ability to control tumor progression is still unknown. Here, we describe how accumulation of specific lipids in areas of the tumor microenvironment populated by CD8+ T cells drives their exhaustion in pancreatic ductal adenocarcinoma. We found that intra-pancreatic CD8+ T cells progressively accumulate specific long-chain fatty acids, which impair their mitochondrial function and trigger major transcriptional reprogramming of pathways involved in lipid metabolism. In particular, intra-pancreatic CD8+ T cells specifically down-regulate the very long-chain acyl-CoA dehydrogenase (VLCAD) enzyme, which exacerbates accumulation of long- and very long-chain fatty acids leading to lipotoxicity. Our results demonstrate that, during cancer progression, compositional changes of the microenvironment influence the anti-tumor response and this is due, at least in part, to the lack of metabolic flexibility of tumor infiltrating CD8+ 18 T cells. Poor persistence and progressive loss of function of T cells limit the efficacy of adoptive T cell therapy for the treatment of pancreatic cancer. Our study paves the way for improving the efficacy of immunotherapy based on the metabolic characterization of the tumor microenvironment and on the metabolic reprogramming of tumor-specific T cells, which may overcome one of the major hurdles to immunotherapy for pancreatic cancer

Keywords: Tumor microenvironment, CD8+ T cells, Long-chain fatty acids, ACADVL.

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## B107 / Analysis of bone marrow CD8 T cells from pediatric leukemia patients reveals targets for T cell engineering: Cancer immunotherapy tailored to the leukemic micromilieu

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Introduction: Acute leukemia is the most common malignancy in children. Despite recent therapeutic advances, patients with relapsed or refractory disease have a dismal prognosis and require new treatment strategies. T-cell immunotherapy and especially chimeric antigen receptor (CAR) T cells against CD19+ acute leukemia have revolutionized treatment of those high-risk patients. Recent data, however, suggests that despite initial complete response rates of about 90% in patients treated with CD19 directed CAR T cells, relapse rates of more than 50% occur over 24 months (1). Those relapses do often result from leukemia CD19 antigen loss or insufficient CAR T cell persistence (2). We hypothesize that leukemia itself negatively affects T cell function as immune escape mechanism. Thus, we set out to analyze T cells from pediatric acute leukemia bone marrow samples and age-matched healthy controls by flow cytometry, RNA-Seq and ATAC-Seq. The overall aim of this study is to identify relevant genes for T cell engineering in order to render them fit for serial leukemia killing within the micromilieu of leukemic bone marrow.

Methods: We analyzed bone marrow samples from pediatric patients with B-precursor ALL (BCP-ALL), T-precursor ALL (TCP-ALL) and acute myeloid leukemia (AML) in comParison to healthy bone marrow donors (HD). Cryopreserved bone marrow samples (BCP-ALL: 18, TCP-ALL: 23, AML: 36, HD: 23) were identified in our local biobank. We performed flow cytometry and next generation sequencing (RNA-Seq and ATAC-Seq) of sorted CD8 T cells. We assayed T cell maturation by CD62L, CD95 and CD45RO surface expression and T cell exhaustion by TIM-3, CTLA-4 and LAG3 expression. Results: First, we performed unsupervised clustering of RNA-Seq and ATAC-Seq data of sorted CD8 T cells asking whether CD8 T cells from leukemia patients differ in their transcriptome and chromatin structure from healthy counterparts. Strikingly, principal component analysis (PCA) of RNA-Seq data documented segregation of CD8 T cells from healthy donors, AML patients and BCP-ALL patients. PCA of ATAC-Seq data did document segregation of BCP-ALL CD8 T cells from HD counterparts, while AML CD8 T cells did not cluster separately from HD. Finally, integration of RNA-Seq and ATAC-Seq yielded improved

segregation of three populations: AML, BCP-ALL and HD. Next, we observed increased T cell maturation in patients with acute leukemia by flow cytometry. In particular, frequency of naïve T cells decreased while effector memory T cells increased in leukemic bone marrow compared to HD. T cells from acute leukemia patients expressed higher levels of TIM-3, CTLA-4 and LAG3 by flow cytometry and RNA-Seq. Moreover, significantly increased expression of many elements of the cytotoxic granule machinery by RNA-Seq (among others: GZMB, GZMH, PRF1, CTSD) mirrored the shift towards effector T cells. Finally, we observed lower expression of central genes regulating CD8 T cell proliferation, longevity and memory formation in leukemia patient CD8 T cells, e.g. key CD8 T cell transcription factors and interleukin receptors.

Conclusion: We demonstrate a profound shift towards mature effector T cells in leukemic bone marrow by flow cytometry and RNA-Seq compared to healthy controls. Moreover, we identify multiple key genes of CD8 T cell function differentially expressed in acute leukemia and healthy donors. We hypothesize that engineering those genes will enable us to improve T cell engineering for leukemia immunotherapy by modulating e.g. T cell proliferation and sustained cytotoxicity. In summary, we want to develop adoptive T cell therapies resistant to exhaustion induced by leukemic micromilieu.

Keywords: pediatric oncology, T cell exhaustion, cancer immunotherapy, T cell engineering.

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# TME analysis

## B108 / Plasma-derived exosomes in head and neck squamous cell carcinoma patients as potential biomarkers of response to immune therapies

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Background: Circulating exosomes play a key role in immune suppression and disease progression. To evaluate their role as biomarkers of response to immunotherapy, we monitored changes in the cargo of exosomes isolated from plasma of patients with head and neck squamous cell carcinoma (HNSCC) treated with Cetuximab, Ipilimumab and IMRT.

Methods: Patients (n=18) with advanced HNSCC enrolled in the phase I clinical trial (NCT01935921) donated plasma at baseline and during immunotherapy (weeks 5, 14). Exosomes were separated into T cell-derived and non-T cell-derived exosomes by immunoaffinity capture. On-bead flow cytometry was used for detection of CTLA-4, PD-L1 and CD15s (Treg marker) on exosomes. To immunocapture tumor-derived exosomes (TEX), a microarray containing 4 antibodies specific for antigens overexpressed on HNSCC was used. results were correlated to patients' outcome.

Results: All patients had high TEX levels at baseline with a decrease at week 5. However, in patients who recurred (n=4), TEX levels increased at week 14; in contrast, TEX levels in 14 patients responding to therapy remained low. PD-L1 levels in CD3(-) exosomes (TEX enriched) were elevated at week 5 in patients with recurrence but significantly decreased in responders. The responders had significantly higher levels of CTLA-4 in exosomes at baseline than non-responders. However, a drastic decrease of CTLA-4 occurred during therapy. Levels of CD15s were elevated at week 5 in all patients, with normalization at week 14 in disease-free patients only.

Conclusion: Exosomes in plasma of cancer patients treated with immune therapies may serve as biomarkers of early response to treatment.

Keywords: Head and Neck Cancer, Biomarker, Exosomes, Immune therapy.

## B109 / Identification of a DC1 immuno-regulatory module that is associated with tumor antigen uptake and partially driven by TAM receptor AXL

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Checkpoint blockade therapies have dramatically improved can-

cer care, but a large subset of cancer patients fails immunotherapy regimens. While conventional dendritic cells (DC)1 and DC2 are found in tumors, DC1 were shown to control tumor response to checkpoint blockade in preclinical models and are associated with better overall survival in cancer patients, reflecting their specialized ability to prime CD8+ T cell responses (1, 2). Paradoxically, DC1 can also be found in tumors that resist checkpoint blockade, suggesting that, like many tumor infiltrating T cells, DC1 functionality may be altered in tumors.

To address this question, we performed scRNAseq analyses to characterize DC gene architecture in human and mouse non-small cell lung cancer (NSCLC) lesions. We identified three intratumoral DC clusters; the canonical DC1 and DC2 populations, as well as a third DC cluster that expressed many immuno-regulatory genes (Cd274, Pdcd1lg2, Cd200, Aldh1a2 and Fas), maturation and activation genes (Cd40, Ccr7, Cd83 and Il12b) and almost no Toll-like receptor genes. We named this cluster "mature DC enriched in immuno-regulatory molecules" (mregDC). The use of Cellular Indexing of Transcriptomes and Epitopes by sequencing (CITEseq) analyses revealed that many mregDC genes were expressed by DC1 and DC2 upon capture of tumor antigens, while some were specific to tumor-charged mregDC1 such as Il12b. Ex vivo, mregDC1 had the capacity to induce both regulatory T cells as well as interferon gamma producing antigen-specific CD8+ T cells, underlining DC1 capacity to process and cross-present tumor antigens and induce antigen-specific CD8+ effector T cell responses, but at the same time the capacity to induce regulatory T cell responses. These results emphasize the dual regulatory and immunogenic program of DC1 and further highlight the need to examine whether modulation of the regulatory program could enhance DC1 functionality in situ.

To identify drivers of the mregDC program that could lead to DC1 immuno-regulation upon tumor antigen uptake, we probed the contribution of the pathways known to regulate PD-L1 and IL-12 induction. Further analysis revealed that PD-L1 upregulation upon tumor antigen uptake in mregDC1 occurred even in the absence of type I and type II interferon, MyD88/TRIF and inflammasome signaling, while IL-12 production by DC1 was critically dependent on interferon gamma signaling in vivo.

Phagocytic receptors are known to contribute to immuno-modulation in both macrophages and DC (3), prompting us to assess their effect on DC1 undergoing antigen uptake. We found that TAM receptor AXL was upregulated in DC1 upon tumor antigen uptake and that PD-L1 upregulation was partially dependent on AXL, as mice transplanted with bone marrow deficient in AXL show reduced upregulation of PD-L1 on mregDC.

Here we uncovered a regulatory module associated with tumor antigen uptake that reduced DC1 functionality in tumor lesions and suggest that targeting the mregDC module can significantly enhance therapeutic anti-tumor immunity. These results also suggest that therapies that increase tumor cell death and tumor antigen release could benefit from the modulation of the DC1 regulatory program.

Keywords: Dendritic cells, Immuno-suppression.

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## B110 / Single-cell RNA sequencing identifies variations in immune cell phenotype within pediatric ependymoma subtypes

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Ependymoma (EPN) is an aggressive childhood brain tumor with poor survival. Most pediatric EPN occur in the posterior fossa (PF) and are classified as PFA1 or PFA2. We have previously shown these subgroups have distinct immune phenotypes. PFA1 tumors, which do poorly, have chronic IL-6 secretion that we have shown pushes the immune microenvironment to an immune suppressive phenotype. In contrast, PFA2 tumors have a more immune activated phenotype and have better outcome. The objectives of this study were to 1. use single-cell(sc) RNAseq to identify and more descriptively characterize the infiltrating immune cells and 2. use the database generated to determine the genomic effects of immunotherapy trials in pediatric PF EPN. Using the 10x Chromium Drop-seq platform, we analyzed approximately 8500 cells from 21 posterior fossa EPN patient samples. We used advanced machine learning techniques to identify distinct myeloid (dendritic and monocytes) and lymphoid (T-, NK-, and B-cells) sub populations within both PF EPN subgroups. The monocyte cells were difficult to interrupt as the data suggested a continuum of phenotypes exist within PFA1 and PFA2. Through lineage tracing of the monocytes we were able to tease out that consistent with our prior hypothesis, PFA2 monocytes expressed more genes associated with an anti-viral response, while PFA1 monocytes had genes associated with an immune suppressive phenotype. These distinct phenotypes also correlated with significantly more T-cell infiltration in PFA2 than PFA1. Recently we found in samples from a large clinical trial for pediatric EPN, T-cell infiltration was correlated significantly with outcome. We have recently begun trials using combination GM-CSF and trastuzumab in relapsed PF EPN. Patients were treated with GM-CSF and intrathecal administration of trastuzumab prior to resection. RNAseq analysis on bulk tumor showed direct evidence of immunophenotype reprogramming in response to GM-CSF, with many of the genes upregulated consistent with those found in the PFA2 scRNAseq dataset, ie MHC class II genes and genes associated with T-cell activation. We also observed downregulation of genes associated with immune suppression, like CD163 and CTLA4. These studies articulate the need to understand the biology of EPN in terms of its subtypes in order to move forward with immunotherapies. EPN subtypes are different enough that it is a distinct possibility that different immunotherapy strategies will be needed for each subtype.

Keywords: ependymoma, single-cell RNAseq, pediatric.

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## B111 / Single-cell RNA sequencing reveals immune dysfunction in the gastric cancer tumor microenvironment and reprogramming from normal tissue

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The tumor microenvironment (TME) consists of heterogenous cell types. These features are masked by bulk Methods of analysis but can be revealed with single-cell RNA sequencing (scRNA-seq). We hypothesized that scRNA-seq analysis of gastric cancer together with paired normal tissue and PBMCs could reveal the dysfunction in its TME.

Surgical resections or biopsies were dissociated into single cell suspensions from seven patients with gastric cancer and one patient with intestinal metaplasia, together with their paired normal tissue. PBMCs were isolated from two patients. Cell suspensions were subjected to scRNA-seq (10X Genomics). Cell-gene count matrices (10X Cellranger) were normalized and subjected to principal component analysis, graph-based clustering and dimensionality reduction (Seurat). Cell lineages were assigned using literature-based markers. Gene regulatory networks per cell were constructed to identify 'regulons' (SCENIC) together with pseudotime trajectories (Monocle). Receptor-ligand interactions were determined between cell lineages (CellphoneDB). ComParison to published gene sets was performed using Gene Set Variation Analysis (GSVA) for each cell. Multiplex immunohistochemistry (IHC) (Ultivue Inc.) was performed on formalin fixed tissue.

We sequenced 32407 single cells from tumors or metaplasia, 18657 single cells from paired normal tissue and 5103 PBMCs with an average sequencing depth of 59880 reads/cell. We identified various lineages including epithelium, fibroblasts, endothelium, T and B lymphocytes, macrophages, mast cells and neuroendocrine cells. Tumors were significantly enriched for fibroblasts, endothelial cells, pericytes and macrophages compared to paired normal mucosa. TME-exclusive stromal cells had differences in the gene expression program encoding ECM constituents and secreted factors with differential enrichment of regulons. Macrophages in the TME had six distinct cell states that did not conform to the M1/M2 differentiation paradigm, with a diverging trajectory compared to PBMC monocytes. TME contained activated dendritic cells, which were absent in normal tissue or PBMCs, with expression of CD83, CCL22, CCL19 and IDO1. Cytotoxic T cells in the TME consisted of one naïve cell subset with low cytotoxicity and two effector subsets with low cytotoxicity and high exhaustion, together with upregulation of multiple immune checkpoint molecules. We detected a small subset of proliferating effector CD8 cells in the TME suggestive of an active immune response that had significantly higher expression of TNFRSF18 (GITR). CXCL13 expressing CD4 and CD8 T cells, associated with neoantigen recognition, were exclusive to the TME. We confirmed presence of CD3, CD45RO and PD1 expressing cells using multiplex IHC in a subset of tumors. TME specific CD8 T cells had overlapping trajectories with those in normal tissue but were distinct from PBMCs. Regulatory T cells were significantly enriched in the TME compared to normal tissue with upregulation of several checkpoint and costimulatory molecules. Each of the TME exclusive T cell subsets revealed enrichment of distinct regulons, indicative of a specific epigenetic profile. NK cells expressed cytotoxic molecules indicative of an anti-tumor immune response but also expressed multiple checkpoints. Plasma cells in tumors expressed IgGs unlike IgAs in normal tissue. Receptor-ligand analysis identified a TME-specific interactome with numerous autocrine and paracrine interactions between all cell types. This included 19 cytokines and their corresponding receptors that can influence immune cell fates.

Our results indicate that gastric cancer is accompanied by remodeling of the normal mucosal microenvironment. This occurs by differences in cell numbers, cell states and inter-cellular interactions. The characterization of the TME landscape in gastric cancer facilitates the understanding of tumor biology and enables the identification of novel immunotherapy targets.

Keywords: scRNA-seq, gastric cancer, TME.

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## B112 / Individual immune activation of local and systemic immune responses determines different pathological and therapeutic effects of neoadjuvant chemotherapy in breast cancer

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Purpose: Activation of the immune response, including T lymphocytes, natural killer (NK) cells, and tumor microenvironmental factors (TMEFs), plays an important role in inducing a therapeutic effect after neoadjuvant chemotherapy (NAC) in breast cancer. However, how an individual patient´s immune response is involved in different pathological and therapeutic effects in breast cancer after NAC, as well as the role of TMEFs in the pathological response, is still unclear. We examined immune activation of systemic and local immune responses to different pathological and therapeutic effects of NAC in breast cancer.

Patients and Methods: From January 2013 to June 2018, 38 patients with stage II–III breast cancer received NAC with anthracyclines and taxanes followed by surgery. Pathological and therapeutic effects were evaluated according to histopathological criteria for the assessment of therapeutic effects outlined by the Japanese Breast Cancer Society. A systemic immune response was evaluated by measuring peripheral (p)NK cell activity, and peripheral CD4 to CD8 (pCD4/8), neutrophil to lymphocyte (NLR), and lymphocyte to monocyte (LMR) ratios, before and after NAC. Peripheral NK cell activity was measured by chromium release assay. The local immune response was evaluated by assessing levels of 14 TMEFs: CD4, CD8, NK, forkhead box protein P3 (FOXP3), cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed cell death 1 (PD-1), programmed cell death ligand 1 (PD-L1), interleukin (IL)-2, IL-6, IL-12, interferon (IFN)-γ, IL-10, transforming growth factor (TGF)-β, and vascular endothelial growth factor (VEGF); using next-generation sequencing in formalin-fixed paraffin-embedded sections collected from preoperative vacuum-assisted biopsy samples and surgical specimens. Results: Therapeutic outcomes were as follows: G1a (N = 8), G1b (N = 13), G2a (N = 7), G2b (N = 4), G3 (i.e. complete; N = 6). In univariate analysis, a G2 or better therapeutic effect was significantly associated with higher NK levels, increased IL-6 and decreased VEGF levels, and potentially associated with higher CD4 and CD8 after NAC. Multivariate analysis revealed a G2 or better therapeutic effect was significantly associated with higher NK levels after NAC (odds ratio [OR] = 1.07; 95% confidence interval [CI], 1.00–1.14; P = 0.0255). In univariate analysis, the disappearance of axillary lymph node metastasis (Ax+) was significantly associated with increased pNK cell activity and higher NK levels, as well as a decreased VEGF level after NAC, and potentially associated with higher CTLA-4 in regulatory T cells (Tregs) before NAC. A decreased NLR was significantly associated with the disappearance of Ax+, increased NK, decreased VEGF, and potentially associated with increased CD8 after NAC. Multivariate analysis showed a decreased NLR was significantly associated with the disappearance of Ax+ (OR = 0.1084; 95% CI, 0.0168–0.6984; P = 0.0194). An increased LMR was significantly associated with higher pNK cell activity after NAC, and higher IL-2 and IL-10 levels before NAC. With subset analysis of 16 patients, an increased reduction rate in pCD4/8 was significantly associated with increased pNK cell activity and a decreased NLR in univariate analysis.

Conclusion: Post-NAC increases in NK cells are critical for the clinical benefit of NAC in collaboration with increased CD4 and CD8, and decreased CTLA-4 and VEGF transcript levels, by coordinating with the release of an immunosuppressive network in response to VEGF, and CTLA-4 in Tregs in the tumor microenvironment. A decreased NLR and pCD4/8, and increased LMR as well as increased pNK cell activity after NAC, may improve the elimination of metastatic tumor cells in axillary lymph nodes in patients with breast cancer. Immune activation, including local and systemic immune responses, as well as a release from immunosuppression, is necessary for the eradication of breast cancer cells after NAC regardless of tumor subtype.

Keywords: Breast Cancer, Neoadjuvant chemotherapy, Immune respoonse, Tumor microenvironment.

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## B113 / Tumor endothelial up-regulation of IDO1 limits the response to CD40-stimulating immunotherapy

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CD40-stimulating immunotherapy elicits potent anti-tumor responses by activating dendritic cells and enhancing T-cell priming, but it can also affect other cell types in the tumor microenvironment. Here, we have investigated how tumor endothelial cells respond to CD40-stimulating immunotherapy in B16.F10 melanoma by isolating endothelial cells from agonistic CD40-antibody- or isotype-treated mice followed by RNA-sequencing. Gene set enrichment analysis revealed an increase in interferon (IFN)-related responses in tumor endothelial cells following CD40-stimulating therapy. The immunosuppressive enzyme indoleamine 2, 3-dioxygenase 1 (IDO1) was predominantly expressed in endothelial cells, and it was up-regulated upon CD40-stimulating therapy. IDO1 expression in tumor endothelium was positively correlated to T-cell infiltration and to an increased intratumoral expression of IFNγ. In vitro, endothelial cells up-regulated IDO1 in response to T-cell-derived IFNγ, but not in response to CD40-stimulation. Combining agonistic anti-CD40 therapy with the IDO1 inhibitor epacadostat delayed tumor growth in B16.F10 melanoma, associated with increased activation of tumor-infiltrating T-cells. Hereby, we have uncovered an immunosuppressive feedback mechanism, in which tumor vessels limit the efficacy of cancer immunotherapy by up-regulating IDO1 in response to T-cell activation.

Keywords: endothelial cells, IDO1, CD40, Immunotherapy.

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## B114 / Targeting SMAD4 mutant SCC microenvironment

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SMAD4 chromosomal loss is frequently in tobacco-associated head and neck cancer, mostly squamous cell carcinomas (SCCs). Development of therapeutic interventions effectively targeting these tumor microenvironment (TME) characteristics is hindered by limited SCC models with metastatic potential in an immune competent Background. To this end, we have created a mouse model in which Smad4 is deleted (Smad4-/-) in stratified epithelia. Smad4 loss causes SCC and metastasis, and compensatory TGFβ1 overproduction. Our findings are validated by The Cancer Genome Atlas (TCGA) analysis showing genetic SMAD4 loss inversely correlated with TGFβ1 levels. Using CytoF, we profiled tumor associated leukocytes Smad4-/- SCCs, and found that different from other tumor types in which TGFβ1 induces "immune cell exclusion" (cold tumors), Smad4-/- SCCs had numerous infiltrated leukocytes including T cells (hot tumors). Among CD45+ cells, myeloid cells, including macrophages and putative myeloid-derived suppressor cells (MDSC), are the highest subpopulations. To dissect the contributions of tumor-associated macrophages (TAMs), we depleted TAMs with liposome clodronate. TAM depletion reduced tumor growth but not metástasis. Because TAMs and MDSCs are the major source of TGFβ1 in tumor microenvironment, we treated Smad4-/- SCCs with a TGFβ-receptor small molecule inhibitor. TGFβ inhibition modestly reduced tumor growth in immune-competent mice but not in immune-compromised mice, suggesting that the therapeutic effect of TGFβ inhibition on tumor growth is T-cell dependent. However, TGFβ inhibition reduced SCC lung metastasis in athymic recipients, suggesting it also targets non-T cells. Further, we found that reduced metastasis correlated with reductions in CD11b+Ly6G+ cells but not F4/80+ cells, suggesting that MDSCs, but not TAMs, contribute to metastasis. We also found that PD-L1 positive cells in Smad4-/- SCCs are mainly myeloid cells, not tumor epitelial cells. A short-term TGFβ inhibition was sufficient to sensitize SCCs to anti-PD-L1-mediated tumor eradication. Taken together, our studies suggest that attenuating a TGFβ-induced immune suppressive and inflammatory TME in Smad4 mutant HNSCCs makes immunotherapy more effective.

Keywords: head and neck cancer, TGFb, SMAD4, PD-L1.

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## B115 / Using ablative radiation to enhance the delivery of antibody for immune checkpoint blockade therapy

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Studies have shown that radiation can augment the efficacy of immunotherapy through different mechanisms. The mechanism is likely protocol dependent. Using murine orthotopic pancreatic ductal adenocarcinoma (PDAC) model, UN-KC-6141, and flow cytometry and immunohistochemical staining (IHC) assays, we demonstated that the protocol of single high dose of radiation treatment (25 Gy) (SHD-RT) could cause more CD8+ T cell infiltration than hypofractionated radiation treatment protocol (5 x 10Gy/fraction) (HF-RT). In addition to the difference on cell infiltrating profiles, the examination of tumor microenvironments (TME) revealed a significantly increase of vessel permeability, as evidenced by the increase of Hoechst33342 dye and dextran distribution, in a time and dose dependent process. Choosing the best combination window, 5 days in this tumor model, results clearly illustrate that more anti-PD-L1 antibody could be delivered to SHD-RT-irradiated tumor than HF-RT-irradiated tumor. The combination of anti-PD-L1 immune checkpoint blockade therapy (ICBT) with SHD-RT has more significant effect on tumor growth delay than the combination with HF-RT protocol. Splenocytes isolated from surviving mice show specific cytotoxicity against UN-KC-6141 cells, but not other unrelated tumor cells. This study demonstrates that a critical time for administrating ICBT agent following SHD-RT is essential to achieve best therapeutic effect.

Keywords: immune checkpoint blockade, tumor microenvironment, radiation therapy.

## B116 / CD86+ antigen-presenting B cells are increased in solid cancers and induce tumor antigen-specific T cell responses

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B cell effector functions do not only include secretion of antibodies, but also presentation of antigen to T cells. Recently, a physiological B cell subset with strong immunostimulatory properties was described in humans. These antigen-presenting B cells (BAPCs) are characterized by a high expression of CD86 and downregulation of CD21. BAPCs are expanded following vaccination or under inflammatory conditions. We analyzed seven different tumor entities for the presence of BAPCs by flow cytometry and found increased percentages in lung adenocarcinomas, head and neck squamous cell carcinomas, hepatocellular carcinoma, breast adenocarcinoma, urethral carcinoma, colorectal cancer and esophageal-gastric cancers. These increased percentages of BAPCs were correlated with increased numbers of tertiary lymphoid structures in the tumor microenvironment. Tumor antigen-specific B cells isolated from tumor-draining lymph nodes of cancer patients showed increased percentages of BAPCs. Furthermore, we demonstrated a strong induction of tumor-specific T cell responses by autologous BAPCs using an antigen-specific fluorospot assay. Our results highlight the relevance of BAPCs as professional antigen-presenting cells in cancer.

Keywords: B cells, Antigen presentation.

## B117 / Patterns of immune checkpoint expression by primary tumor cells and tumor infiltrating lymphocytes across different tumor entities

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Immune-checkpoint inhibition (CKI) demonstrated breakthrough therapeutic efficacy in several kinds of cancer. These therapies are unique, as the primary target is not the tumor cell itself, but the crosstalk between immune system and cancer cells within the tumor microenvironment. Hence, analysis of the respective protein in the tumor microenvironment only partially predicts response to the treatment. For example, recent publications demonstrated that efficacy of anti-PD(L)-1 treatment is not limited to patients with expression of the respective ligand on tumor cells but expression of PD-L1 on tumor-infiltrating lymphocytes (TILs) can be of similar importance.

In this study, expression patterns of 30 described immune regulatory molecules were analyzed on T, B and NK cells in peripheral blood and single cell suspensions of 135 primary tumor samples of 10 different tumor entities using flow cytometry. Expression of the respective ligands and 10 key genes associated with antigen processing was assessed by NanoString technology. Finally, expression of selected ligands on primary tumor cells was assessed by immunohistochemistry (IHC) analysis of tissue microarrays.

Our analyses revealed distinct patterns of TILs in the tumor microenvironment of different origins. While a fraction of lymphocytic subsets showed a large variety across the 10 entities (e.g. B cells), others were similarly increased across all included types of cancer (e.g. regulatory T cells). The majority of the analyzed immune regulatory molecules were overexpressed on at least one TIL subset. For some molecules we also detected overexpression in PBMCs of cancer patients compared to PBMCs of healthy controls. In addition to their expression on TILs, we analyzed expression of immunoinhibitory ligands on tumor cells. Interestingly, the number of expressed immunoinhibitory ligands showed a large variety between the different tumor types. For example, the majority of colorectal cancer patients expressed more than 5 ligands simultaneously, while such co-expressions were rare in renal cell carcinoma samples. As an additional mechanism of immune escape, altered expression of genes associated with antigen processing and presentation was observed in many tumor samples.

Taken together our data provides a comprehensive picture of immune escape across different tumor types. Our data clearly demonstrates that immune escape is a common feature of cancer, but shows remarkable differences between tumors from different primary locations. The expression patterns described in this study are of immediate translational relevance for ongoing and future immunotherapeutic trials.

Keywords: Immune checkpoints, Immune escape, Tumor-infiltrating lymphocytes, Tumor microenvironment.

## B118 / Clonally expanded T cells reveal immunogenicity of rhabdoid tumors

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Rhabdoid tumors (RT) are genomically simple pediatric cancers driven by the biallelic inactivation of SMARCB1, leading to SWI/SNF chromatin remodeler complex deficiency. Comprehensive evaluation of the immune infiltrates of human and mice RTs, including immunohistochemistry, bulk RNA sequencing and DNA methylation profiling studies showed a high rate of tumors infiltrated by T and myeloid cells. scRNA and TCR sequencing highlighted the heterogeneity of these cells and reveled therapeutically targetable exhausted effector and clonally expanded tissue resident memory CD8+ T subpopulations, likely representing tumor-specific cells. Checkpoint blockade therapy in an experimental RT model induced the regression of established tumors and durable immune responses. Finally, we show that one mechanism mediating RTs immunogenicity involves SMARCB1-dependent re-expression of ERVs and interferon-signaling activation.

Keywords: Rhabdoid tumor, Immunotherapy, Single cell RNA sequencing, Endogenous retrovirus.

## B119 / The function and regulatory balance of CXCR5+ tumor infiltrating lymphocytes in human breast cancer-associated tertiary lymphoid structures

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Background: The association between tumor infiltrating lymphocytes (TIL) and positive clinical outcomes in breast cancer (BC) is now sufficiently robust that recommendations were recently made to include this information in the routine pathology reports (St. Gallen breast cancer guidelines 2019)[1]. Previous work from our laboratory demonstrated that TIL can organize in tertiary lymphoid structures (TLS) in BC-associated stroma[2]. We further showed that CXCL13, produced by specialized CD4+ T follicular helper (Tfh) cell, is an important TLS chemoattractant and associated with positive clinical outcomes[3]. The current study investigated how TLS functionally and regulation contributes to immune responses in BC.

Methodology: We prospectively collected fresh primary BC tissues and prepared enzyme-free homogenates to produce TIL suspensions and tumor supernatants for flow cytometry and cytokine/chemokine/immunoglobulin (Ig) analysis, respectively. Matching formalin-fixed paraffin-embedded tumor tissues were analyzed using dual immunohistochemistry and immunofluorescence confocal microscopy.

Results: We show that the CXCL13 receptor, CXCR5, is expressed on subpopulations of CD4+ (Tfh) and CD8+ T cell TIL as well as the majority of TIL-B, with all CXCR5+ TIL co-localizing in TLS. The functional activities of Tfh TIL, assessed using an in vitro assay with allogeneic human splenic B cells, reveals that PD-1hiICOS+ Tfh TIL (in some triple negative and HER2+ but not luminal BC) can provide help to TIL-B for Ig production, also producing high levels of IFNg in the culture. This observation is strengthened by our data showing: 1) a correlation between functional (PD-1hiICOS+) Tfh TIL densities and IgG concentrations in primary BC supernatants; 2) a strong correlation between PD-1+ Tfh TIL and Ki67+ TIL-B in BC-associated TLS; and 3) cell-to-cell contact between Tfh TIL and TIL-B in TLS with active germinal centers. PD-1hiICOS+ (functional) and PD-1lo/intICOS- (non-functional) Tfh TIL were sorted for mRNA analysis with functional Tfh TIL expressing higher levels of IL-21, IFNg and CXCL13. Higher IFNγ expression by PD-1hiICOS+ Tfh TIL suggests they are Th1 and cytotoxic oriented functionally. CXCR5+ T follicular regulatory (Tfr) TIL were also detected in TLS and shown to express, CD25, demethylated FOXP3 and GARP, a marker of active TGFβ. The ratio between Tfh and GARP+ Tfr TIL found that when the balance favors Tfh TIL IgG production is higher. Multiplex IHC was used to identify the positioning of CXCR5+ TIL subpopulations and identified important cell-to-cell contacts associated with active TLS.

Conclusion: Our data show that Tfh TIL subpopulations play major roles in the functionality of BC-associated TLS. The balance between functionally active and

Keywords: CXCR5+ Tumor infiltrating lymphocytes, Tertiary lymphoid structure, Human breast cancer, Function and regulatory balance.

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## B120 / Deciphering the tumor immune microenvironment of HPV-negative and -positive oropharyngeal tumors

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Strong causal etiology has been described between infection with high-risk human papilloma virus (HPV) and cancer of the cervix, penis, vulva, vagina and the oropharynx. We recently demonstrated that HPV-associated oropharyngeal squamous cell cancer (OPSCC) is a distinct clinical entity with a much better prognosis after (chemo)radiotherapy than HPV-negative OPSCC, especially in patients with a concomitant intratumoral HPV16-specific and type-1 cytokine oriented T cell response. To study whether HPV16+ OPSCC patients display a different immune contexture that contributes to a better response of these tumors to standard-of-care therapy, we performed extensive in-depth analysis of immune infiltrates in the tumor microenvironment (TME) of HPV-negative and HPV16+ OPSCC tumors using mass cytometry (CyTOF) and flow cytometry (FACS) analysis, multispectral immunofluorescence analysis, as well as single cell RNA and TCR sequencing analysis.

HPV16 status determination by GP5+/6+ PCR and p16ink4a immunohistochemistry staining, and multispectral three and six color immunofluorescence staining was done on archived formalin-fixed paraffin-embedded tumor tissue. FACS, CyTOF and paired single cell RNA and TCR sequencing analyses of the tumor immune microenvironment were performed on cryopreserved freshly isolated tumor tissues. HPV16 E6/E7-specific T cell reactivity within HPV16+ tumors was determined by proliferation of IL-2-expanded tumor infiltrating lymphocyte (TIL) batches.

Analysis of the TME revealed clear differences in immune cell composition of HPV16-positive and immune response-positive (HPV16+IR+), HPV16+ and immune response-negative (HPV16+IR-) and HPV-negative OPSCC tumors. Whereas HPV16+IR- tumors were strongly infiltrated with B cells, HPV16+IR+ tumors displayed infiltration with CD4+CD161+ and CD8+CD103+CD161+/- T cells with a highly activated CD38+, HLA-DR+ and/or PD-1+ effector (memory) phenotype. To gain insight into the function of these T cells we are currently applying paired single cell RNA and TCR sequencing analysis on CD3-isolated cells, the results of which will be presented. Interestingly, inflamed HPV16+ OPSCC tumors with ongoing HPV16-specific T cell responses were co-infiltrated with activated, Tbet-expressing CD4+Foxp3+ immune suppressive regulatory T cells (Tregs) as determined by quantitative DNA methylation status analysis of the Foxp3 gene locus and functional analysis. Moreover, strong T cell infiltration was accompanied by a fairly unknown population of CD14-CD33-CD163+ myeloid cells, which appeared to be genuine DCs, as demonstrated by FACS and six color immunofluorescence VECTRA staining. These CD14-CD33-CD163+ DC produce high levels of IL-12 and IL-18 and stimulate allogeneic CD4+ and CD8+ T cell proliferation with a similar capacity as the recently defined inflammatory type CD1c+ DC, and were associated with improved survival. Interestingly, in HPV-driven cervical cancer these Foxp3+Tbet+ Tregs and CD14-CD33-CD163+ DCs were also present and positively correlated with HPV16-specific T cell infiltration.

Keywords: tumor immune microenvironment, human papilloma virus, oropharyngeal cancer, intratumoral.

## B121 / B cells and breast cancer : heterogeneity of the plasma cell infiltrate and evolution during tumor progression

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B cells appear to have an important but still controversial role in breast cancer and understanding the cellular and molecular bases of their pro- or anti-tumor roles remains necessary. These opposite functions may be due to the identity of the subsets that infiltrate tumors and their functional properties that are largely dictated by the nature of the cytokines they produce and/or the class of antibody they release. In this respect, anti-tumor antigen antibodies, mostly of the IgG class, are often detected in the serum of cancer patients. By analyzing different cohorts of breast cancer patients using flow cytometry, in situ tissue imaging and analysis of antibodies produced in the tumor microenvironment, we demonstrated that multiple B cell differentiation stages infiltrate invasive breast tumors, with a dominance of memory B cells, naïve B cells and antibody-producing plasma cells, whether or not tertiary lymphoid structures were present. Importantly, plasma cells not only consisted of IgG-, but also of IgA-, and to a lesser extent IgM-, producing cells and localized in both the stromal and tumor areas. Locally produced IgA consisted of both monomeric and dimeric entities and of IgA1 and IgA2 subclasses. Moreover, in situ breast carcinomas, considered as early tumors, had a plasma cell contingent over-dominated by IgA PC while invasive carcinomas presented a significant shift of local humoral immunity toward IgG production, except for a minor fraction of patients, suggesting a differential role of IgG and IgA during tumor progression. These data demonstrate a high diversity of the B cell infiltrate and humoral response in breast tumors and highlight a potential differential role of IgA and IgG plasma cells on tumor progression and patient survival.

Keywords : B cells, antibody-producing plasma cells, breast cancer, ductal carcinoma in situ.

## B122 / Identification and characterization of a natural killer-dendritic cell axis defining checkpoint therapy-responsive tumor microenvironments

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Immunotherapies have been effective in treating cancer patients by boosting T cell responses to tumors, yet 56% of melanoma patients have no objective responses following anti-PD-1 treatment. Here we present work aimed at understanding the role innate immune cells in the tumor microenvironment (TME) play in regulating responses to anti-PD-1 immunotherapy. Type 1 conventional dendritic cells (cDC1s) are a rare subset of DCs found in the TME and are required for T cell-mediated tumor responses in mice. We show that the frequency of cDC1s in the TME of human melanoma patients predicts better overall patient survival and responsiveness to anti-PD-1 immunotherapy. Given the protective effect of cDC1s in the TME we explore the cellular and molecular mechanisms regulating cDC1 abundance in the TME and find that cDC1 numbers in the TME of human melanoma samples correlate with intratumoral expression of the gene encoding the cytokine FLT3LG. Utilizing human samples and a novel Flt3l-reporter mouse we show that natural killer (NK) cells in the TME are the integral cell type that produces FLT3LG and regulates cDC1 abundance in the TME. Our work defines an innate immune axis, consisting of NK cells and cDC1s, that is required for productive anti-tumor immune responses, patient survival, and is the strongest immune correlate with responsiveness to anti-PD-1 immunotherapy. Thus, we further explore the molecular and cellular mechanisms that regulate NK cell abundance and Flt3l production in the tumor and at steady state. Utilizing single cell RNA sequencing, live-imaging, and functional studies of the interactions that lead to delivery of Flt3L to cDC1s, we show that NK cells likely play a broad role in regulating cDC1 levels in animals and uncover an exciting new function of NK cells in cancer. These studies reveal that innate immune cDC1s and FLT3LG producing NK cells in the TME cluster together as the best prognostic tool for patient responsiveness to T cell directed immunotherapy and that these cells are necessary for enhanced T cell tumor responses; suggesting that novel therapies targeting NK cell abundance or activity in the TME may hold promise in increasing the efficacy of anti-PD-1 immunotherapy.

Keywords : dendritic cells, natural killer cells, tumor microenvironment, checkpoint therapy.

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## B123 / Tissue-specific differences in the tumor microenvironment influence responses to immunotherapy

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Investigation into variable response rates to cancer immunotherapies has exposed the immunosuppressive tumor microenvironment (TME) as a limiting factor for therapeutic efficacy. An emerging determinant of TME composition is the anatomical location of tumor growth as clinical data has revealed associations between certain metastatic sites and reduced therapeutic responses, particularly to checkpoint blockade. Preclinical models to study tissue-specific TMEs have eliminated the influence of genetic heterogeneity, but have limited comParisons to subcutaneous and orthotopic models of tumor growth. Therefore, we sought to investigate the TMEs of tumors at common metastatic sites and the tissue-specific impact on αPD-1/αCTLA4 and trimAb (αDR5, α4-1BB, αCD40) immunotherapy responses in the 67NR mouse breast cancer and Renca mouse kidney cancer models. When growing in the lungs, tumors were resistant to both therapies whereas the same tumor line growing in the mammary fat pad (MFP), subcutaneously (SC) or liver were responsive and could be completely eradicated, even though disease burden was greater. Assessment of tumor cells, vasculature and drug delivery in 67NR lung or MFP tumors revealed no major differences and prompted investigation into the immune TME. Resistant lung tumours had a more immunosuppressive TME with increased infiltration of myeloid derived suppressor cells (MDSCs), decreased T-cell infiltration and activation, and decreased NK cell activation. Furthermore, depletion of various immune cell subsets indicated an increased dependence of NK cells in tumor control of lung tumors compared to MFP tumors. Thus, targeting T-cell responses in the lung tumor setting was not sufficient to elicit an anti-tumor response and could explain resistance to trimAb and αPD-1/αCTLA4 immunotherapies. Together, our research demonstrates that tissue-specific TMEs influence responses to immunotherapies and highlights the importance in further defining tissue-specific response patterns in human cancers to optimize current and future immunotherapies.

Keywords : tumor microenvironment, tissue-specific tumor microenvironment, immunotherapy, checkpoint blockade.

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## B124 / Interfering with the tumor immune microenvironment of KRAS mutant lung cancers.

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With the advancement of immunotherapies for lung cancer, it has become apparent how insufficient our knowledge is surrounding the interactions between the tumor and the immune system. In particular in the context of combining immunotherapy with conventional chemotherapy or novel targeted therapies, it is of the utmost importance that we understand the effects that those therapies have on the tumor immune infiltrate and how that would counteract or synergize with immunotherapy. We have used multiplex flow cytometry to characterize the basal tumor immune infiltrate of various spontaneous and orthologous syngeneic mouse models for lung cancer, with different levels of immunogenicity. In addition, using Imaging Mass Cytometry, visualizing and quantifying >35 markers simultaneously in mouse tumor tissue sections, we studied the localization and interactions of the immune cells within and surrounding the tumors. This has highlighted the level to which tumors are regulating their micro-environment, actively excluding all potential effector cells while attracting various myeloid cells. We have further characterized the Tumor Associated Macrophages (TAMs) and interfered with their recruitment, to study their relevance in tumor immune evasion. In addition we are looking into the role of oncogene signaling in the orchestration of the tumor immune microenvironment, by targeting the KRAS signaling pathway in vivo and measuring the changes in tumor immune composition.

Keywords: Lung cancer, Imaging Mass Cytometry, Tumour microenvironment, KRAS.

## B125 / Single cell analysis reveals the pivotal role of the innate immune compartment in aCTLA-4 anti-tumor response.

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Immunotherapies have and continue to revolutionize cancer patient care, however much of our understanding of the mechanism of action of these therapies is currently limited. The accumulation of regulatory T cells (Tregs) in the tumor hampers anti-tumor activity and correlates with bad prognosis in several human cancers. Anti-CTLA4 monoclonal antibodies (mAbs) have been extensively studied, and their activity depends both on the blockade of the CTLA4 co-inhibitory molecule, as well as the intra-tumoral depletion of Tregs, increasing effector cell abundance, and favoring tumor rejection. Fc-gamma receptor (FcgR) co-engagement have been proven to be important for the action of aCTLA4 in addition to its Treg-depleting activity via antibody-dependent cellular cytotoxicity (ADCC), however it is unclear what are the cellular changes involved. Therefore we wished to dissect the differences between aCTLA4 mIgG1, an antibody which only blocks the CTLA4 receptor, and aCTLA4 mIgG2a, which has a dual activity of both blocking the receptor and depleting Tregs. To address this, we performed single-cell RNA-seq of infiltrating leukocytes from tumors in mice treated with aCTLA4 mIgG1, mIgG2a or left untreated (UT). This high-resolution comParison revealed unique cellular profiles generated by each treatment. While the blocking-only mIgG1 was similar to UT, mIgG2a with ADCC effector function demonstrated major changes. Tumors in mIgG2a treated mice showed a rapid decline of immune suppressive macrophages, and the emergence of pro-inflammatory monocytes, as well as NK cells and naïve CD8 T cells. In addition, mIgG2a treated mice displayed a gradual increase of CD4 T cell subsets throughout treatment. Finally, a vast tissue repair signature was observed in later time points of aCTLA4 mIgG2a treatment, comprised of MRC1+ macrophages, neutrophils, and myeloid-derived suppressor cells (MDSCs), while CD8 T cell abundance declined. In summary, our findings provide an in-depth view of the differences in mechanisms of action between an aCTLA4 blocking mAb, and an optimized blocking-depleting mAb, emphasizing how FcgRs co-engagement leads to enhanced anti-tumor response via the innate immune compartment.

Keywords: Anti-CTLA-4, Fc-gamma receptors, Innate immunity, Antibody-dependent cellular cytotoxicity.

References:

## B126 / Identification of two functionally distinct subsets of macrophages infiltrating human breast cancer.

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Tumor associated macrophages (TAMs) infiltration is a hallmark of solid tumors and generally correlates with poor prognosis. However, TAMs cellular diversity remains ill-defined in human tumors. Here we perform an unbiased single cell RNA sequencing approach on breast cancer metastatic lymph nodes (LNs) and primary tumors to characterize TAMs diversity. We identify two major populations of tumor-associated CD14+ cells: S100A8+CCR2+APOE- monocytes and S100A8-CCR2-APOE+ macrophages. Using flow cytometry analysis and immunochemistry, we show that: 1) APOE+ macrophages are enriched at the tumor border within metastatic LNs, 2) S100A8+CCR2+ monocytes are associated to non-metastatic LNs and healthy breast tissues.

APOE+ macrophages can be further subdivided in two populations: 1) APOE+TREM2+ TAMs expressing SPP1, CADM1 and FN1 and 2) APOE+FOLR2+ TAMs expressing SEPP1, MMP9 and SLC40A1. The transcriptional signature of APOE+TREM2+ TAMs negatively correlates with patient survival. By contrast, the transcriptional signature of APOE+FOLR2+ TAMs is a predictor of survival. This suggests that the two TAM populations may have opposite effect on tumor progression. Collectively, our data reveal the diversity of CD14+ cells and TAMs within breast cancer tumors and provide the molecular basis to assess their unique functional properties.

Keywords: Macrophages, Breast cancer, Metastatic lymph nodes, Subsets.

## B127 / Modulation of NK cells maturation and function in tumor microenvironment of Non-Small-Cell Lung Carcinoma

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bonne Universite, Universite Paris Descartes, Paris, France).

Natural killer (NK) cells are among the first line of defense against cancer cells. As such, they play a central role in Non-Small Cell Lung Carcinoma (NSCLC) initiation and metastatic transition. Previous works of the lab demonstrated that NK cells in NSCLC are excluded from tumor nest and enriched in the tumor stroma where they display an exhausted profile, characterized by down-regulation of activating receptors (NKp30, NKp80, CD16, DNAM-1), reduced IFN gamma secretion and impaired capacities of degranulation (CD107a) (1)(2). These alterations are specifically induced by the Tumor MicroEnvironment (TME), since NK cells in non-tumoral paired lung tissue are comparable to blood NK cells. Transcriptomic analyses on highly purified CD3-CD56+ cells revealed that intratumoral NK cells have a specific gene expression signature, with over expression of gene implicated in exhaustion, and strong modulation of gene involved in regulation of cytotoxic functions, and of migration.

Our study now aims at deciphering the specific mechanisms by which TME modifies NK recruitment, activation and functions. Investigations on pre-metastatic stages of NSCLC confirmed the exhausted and immune suppressive profile of intratumoral NK cells. In order to study the switch from active NK cells to exhausted ones at different tumor stages, we developed a novel mice model of orthotopic lung tumor, by intratracheal injection of LLC cells.

We characterized the tumoral immune infiltrate of this model according to lung tumor stages. Both in human and mice lung tumors, NK cells number decreases in the tumor as compared to non-tumoral tissue. This decreased number of NK is coupled with a lower expression of chemokine receptors (such as fractalkine receptor), both at mRNA and protein level. Moreover, in the tumor, the maturation subsets are unequally distributed with a decrease of more mature NK cells (CD3-, NK1.1+, CD11b+) in favor of more immature ones (CD3-, NK1.1+, CD11b-) (3)(4). These data confirm the exclusion of mature NK cells from the tumor nest and suggest a chemokine-dependant mechanism favoring the less mature ones into the tumor. In mice, the conversion between NK and Innate Lymphoid Cells 1 (ILC1) has also been studied, showing the plasticity of the immune cells in the TME. The global population of intratumoral NK cells express higher immune checkpoints such as TIGIT, suggesting lower cytotoxic capacities, strengthened by a lower expression of CD107a. Taken altogether, our data suggest a combined mechanism in which recruitment of immature NK cells seems to be favored by the TME and in parallel, mature NK cells are exhausted, with an increased expression of inhibitory receptors and impaired cytotoxic capacities.

In a near future, the precise expression profile of each subpopulation of NK cells needs to be assessed, migratory and functional assays on these NK cells will be evaluated as well as the capacities of these NK cells to modulate the function of other immune cells in the TME. These experiments will confirm the selective mechanism set up by the TME in order to evade NK cell cytotoxicity, and may open the way to develop novel therapeutic strategies targeting the NK cell compartment.

Keywords: NK cells, TME, Exhaustion.

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## B128 / hMENA regulates the TGF-β-mediated crosstalk between tumor cells and cancer associated fibroblasts

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Reciprocal interactions between cancer cells and cancer associate fibroblasts (CAFs) are crucial in tumor growth and aggressiveness. This communication includes the involvement of paracrine signals mediated by growth factors and cytokines with a high impact on local tumor microenvironment (TME).

The TGFβ signaling is a key regulator of cancer cell-CAF interactions during the initiation and progression of human cancers. Recently the activation of TGFβ in fibroblasts has been associated with T cell exclusion and resistance to immune checkpoint blockade (1, 2), suggesting the relevance of identifying signaling pathways related to TGFβ activated CAF.

CAFs, by producing extracellular matrix components (ECM) and an array of cytokines and growth factors among which TGFβ plays a critical role, regulate tumor growth and progression. Stromal-derived TGFβ can activate resident fibroblast via autocrine signaling and induce the upregulation of paracrine factors which promote signaling pathway activation in adjacent cancer epithelial cells. Reciprocally, TGFβ secreted by epithelial cancer cells exerts a paracrine influence on stromal cells resulting in increased production of ECM key determinant of cancer progression and prognosis. hMENA, an actin-regulatory protein, has been reported by our and other groups as a main player in supporting the malignant transformation and progression of diverse cancers. We have shown that the tissue specific splicing of the hMENA generates two isoforms, the epithelial hMENA11a and mesenchymal hMENAΔv6 (3). The expression of hMENAΔv6, along with the lack of hMENA11a, is crucial for TGFβ-mediated EMT (4), and hMENA isoforms are checkpoints of the β1 integrin-ECM signaling pathway (5), representing prognostic factors in PDAC and early stage NSCLC (4, 5, 6). The aim of this study is to identify a complementary role of hMENA/hMENAΔv6 in CAFs, focusing on TGFβ-mediated crosstalk between cancer cells and CAFs in NSCLC and PDAC. We found that hMENA/hMENAΔv6 overexpression identified a subset of CAFs with pro-tumoral functions in NSCLC and PDAC. The soluble factors produced by hMENA-over-expressing epithelial cancer cells induce the expression of hMENA/hMENAΔv6 in CAFs. By exploring the factors involved in hMENA-mediated cancer cell-CAFs cross-talk we found that the silencing of hMENA/hMENAΔv6 in CAFs significantly decreased the secretion of the three TGFβ ligands (TGFβ1, TGFβ2, and TGFβ3), downregulated the expression of TGFβR1 and 2 and inhibited the TGF-β signaling activation. This in turn, resulted in inhibition of CAF activation and functions, as evidenced by a reduced TGFβ-mediated collagen gel contraction ability and fibronectin- fibrillogenesis. Furthermore, the hMENA/hMENAΔv6 silencing regulates the TGFβ-mediated cytokine/chemokines secretion to affect the paracrine signaling pathway activation in epithelial tumor cells.

Reciprocally, hMENA and its isoform expression in tumor cells specifically regulates the secretion of TGFβ2. hMENA silencing in NSCLC cells impairs the ability of tumor cell secretoma in activating TGFβ signaling in CAFs, as evidenced by the inhibition of pSMAD2 expression.

In conclusion this novel finding provides the evidence for a crucial role of hMENA and its tissue specific splicing program as regulator of TGFβ paracrine signaling in the cross-talk between tumor cells and CAFs. We highlight the importance of inhibiting hMENA-mediated signaling pathways also in view of the new advance of combined immunotherapy and TGFβ inhibitors in the clinical practice. Supported by AIRC IG PN

Keywords: cancer associated fibroblasts, TGFbeta.

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## B129 / Analysis at single cell level of the heterogeneity of tumor associated macrophage in triple negative breast cancer.

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Introduction Tumor associated macrophages (TAM) represent strong regulator of immune suppression, playing an essential role in diverse phases of tumor growth and dissemination. The role of inhibitory/co-stimulatory signals on TAM and their possible implication in the modulation of the outcome of human malignancies remain to be investigated. For these reasons, we aim to dissect the true composition of TAM in human breast cancer.

Methods Here, we analysed at RNA-single cell level the myeloid compartment enriched in triple negative breast cancer (TNBC). After mechanic and enzymatic dissociation of non-tumor (NT) and tumor (T) samples we enriched, by sorting, HLA-DR+CD11c+ myeloid cells from each counterpart, and then performed, by 10X Chromium technology, the RNA analysis at single cell level.

results-Conclusion The bioinformatic analysis of RNA, at single cell, demonstrated a peculiar distribution of different clusters between NT and T counterparts.

First, we observed an accumulation of “activated monocytes” in the intermediate states of differentiation, showing a peculiar signature of classical inflammatory monocyte markers like FCGR3A, SOD2, IFITM2, IFITM3, ISG15, MAFB, S100A8, S100A9, CD14 expressing also different adhesion molecule-related genes, ICAM1, PECAM1, ICAM2, suggesting a preferential accumulation at the tumor site.

We could also detect DC populations characterizing by the expression markers like CCR7, CXCR4, CD1A, CD207, AXL, CD80 and again a cluster representing DC2, showing CD1c, CLEC10A, with high expression of different HLA-DR-DQ molecules.

Accumulated at different levels, in different patients, we could also find MRC1, MSR1, TREM2, APOE, FOLR2, identified recently as TAM genes, all shared in a specific subset. Supporting the notions of pro-tumorigenic functions of these genes, the differential distribution among patients may impact the overall immune responses to cancer.

Further evaluations will be focus on the functional study of diverse populations, potentially dissecting pro- or anti-tumor roles.

Keywords : Myeloid cells, triple negative breast Cancer, HLADR+CD11C+, single cell data.

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Cancer Cell. 2019 Apr 15 ;35(4):588-602.e10. doi : 10.1016/j.ccell.2019.02.009. Epub 2019 Mar 28. Cell. 2018 Aug 23;174(5):1293-1308.e36. doi : 10.1016/j.cell.2018.05.060. Epub 2018 Jun 28.

## B130 / Single-cell analysis identifies conserved human and mouse tumor myeloid populations that differentially affect glioblastoma progression

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Glioblastoma multiforme (GBM) is an invariably fatal primary malignant brain tumor. The GBM immune infiltrate is recognized as a key player in tumor progression, potentially exerting both pro- and anti-tumoral functions. A better understanding of these cells is imperative for the development of more efficient therapies. We have relied on single-cell RNA sequencing to unravel the complexity of the glioblastoma immune landscape. This showed that GBM tumors contain a highly heterogeneous immune compartment, which was strikingly similar in human and syngeneic mouse glioblastoma tumors. The largest fraction of tumor-infiltrating immune cells were of myeloid origin, consisting of macrophages and conventional dendritic cells. Interestingly, we identified multiple distinct tumor associated macrophage (TAM) subsets, of which the transcriptional signatures were partly driven by their ontogeny. In addition, multi-parametric flow cytometry allowed for cell sorting of the various TAM and cDC subsets, which was followed by an extensive functional profiling. This showed clear differences in T-cell stimulatory and suppressive capacities, phagocytic activity and pro-angiogenic potential, which was driven by TAM ontogeny and differentiation state. Finally, we report a combination therapy that simultaneously targets multiple TAM populations, resulting in improved therapy responses. Together, our data help to unravel the complexities of the GBM immune compartment, paving the way for strategies to optimally “re-educate” specific TAM or cDC subsets to tilt the balance towards tumor eradication.

Keywords : Glioblastoma, Microglia, Tumor associated macrophages, Single cell RNA seq.

## B131 / Quantitative and spatial analysis of single cells in the tumor microenvironment using immunofluorescence protein multiplexing

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The presence and density of various functional immune cell subsets within the tumor microenvironment strongly influence the patient’s response to immunotherapeutic treatment. However, the effectiveness of immunotherapy may be limited due to the heterogeneous spatial distributions of cancer cells and infiltrating tumor lymphocytes (TILs). As such, development of a tool that can distinguish sub-populations of cancer cells and immune infiltrates, and that also enables visualization and measurement of their spatial distributions, will be invaluable for assessing and monitoring patients’ immune and cancer profiles in the planning and delivery of immunotherapy. Here we report the use of an in situ immunofluorescence protein marker multiplexing system (MxIF, Cell DIVE™ GE Healthcare, Issaquah WA)[1] to study cancer and immune markers at cell level and to quantify immune subsets in ovarian cancer using single 4 um sections of formalin-fixed, paraffin-embedded (FFPE) tissue. A panel of immune cell markers including CD3, CD4, CD8, CD68, PD-1, PD-L1, together with proliferative and cancer-specific markers were studied on whole sections of ovarian cancers with MxIF. Cell counts of different immune cell types were quantified by performing thresholding and co-expression analysis. These quantifications were compared to flow cytometric data from the same cases as validation. Moreover, dimensional reduction with t-sne [2] was applied to the high-dimensional MxIF datasets to visualize the presence (or absence) of different immune or cancer cell clusters and to compare these among a set of ovarian cancer cases. We observed significant variations in the densities of immune infiltrates as well as the expressions of the immune checkpoint molecules in these samples. These results are being correlated to clinical information to evaluate whether certain patterns of checkpoint marker expressions on immune subsets may predict response to checkpoint inhibition. In addition to the identification and quantification of various cell types, in situ protein multiplexing also provided spatial information with

respect to proximity and most common neighbor analysis using co-occurrence matrix analysis. We studied the likelihood that the immune checkpoint proteins are in close proximity to each other, to tumor cells or to TILs. We also compared the densities of TILs present in the stromal (sTIL) vs intraepithelial (iTIL) regions. Our work demonstrates the application of MxIF in phenotyping the immune repertoire and in assessing their heterogeneous spatial distributions in tumors.

Keywords: Protein Multiplexing, Tumor microenvironment, spatial analysis, quantitative image analysis.

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## B132 / Spatial and single-cell transcriptome profiling of TIM-3 biology and function in the tumor microenvironment

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Therapeutic blockade of T cell inhibitory receptor, PD-1, often referred to as “checkpoint blockade”, is showing unprecedented results in the treatment of cancer patients. The next generation of targets for checkpoint inhibition includes cell surface receptor TIM-3, revealed as a novel mode of resistance to PD-1 blockade and also expressed on myeloid cells, suggesting a key function extending beyond T cell inhibition. TIM-3 biology in the tumor microenvironment (TME) with respect to spatial compartmentalization of immune cells is a key area of investigation. To determine the intrinsic role of TIM-3 in T cell/myeloid biology within the TME, we designed a Mixed Bone Marrow Chimera mouse model to compare TIM-3 wild-type (WT) and knockout (KO) immune cells in the same host. This model allowed us to assess differences in immune cell phenotype and function due to the intrinsic absence of TIM-3 rather than variation in the host TME. Whole transcriptome profiling of tumor tissue provides transcriptional signatures of the TME and can be further utilized to understand the intrinsic biology behind immune-immune and immune-tumor interactions which are imperative for better therapeutic design. Single cell RNA-sequencing (scRNA-seq) provides expression data for cell populations in the TME but lacks spatial information necessary for cell-cell interaction analysis. The development of spatially resolved transcriptomic techniques enables the integration of both broad and high resolution transcriptome data. Utilizing the Spatial Transcriptomics® assay with integration of 10x Genomics® scRNA-seq, we have assessed the function and expression of TIM-3 in the TME in a spatial context. We have identified distinct inhibitory immune cell signatures that correlate with TIM-3 expression. Furthermore, TIM-3+, TIM-3-, and TIM-3 KO T cell populations displayed unique transcriptional profiles, suggesting that TIM-3 may play an intrinsic role in the development of these unique signatures. The spatial location of these transcriptionally unique populations in the TME provide insight into the cell-cell interactions of inhibitory immune cells. These findings further elucidate the role of TIM-3 in immune cell function within the TME and identify inhibitory immune cell targets for immunotherapy.

Keywords: TIM-3 Biology, Spatial Transcriptomics, single cell RNA-sequencing, Transcriptome Profiling.

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## B133 / Immunoprofiling of tonsillar cancer

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Human papilloma virus (HPV) is recognized as a causative agent of oropharyngeal cancer (OPC) including tonsil cancer. Approximately 70% of tonsil cancers are associated with HPV, and patients with HPV+ tumors have better prognosis, which is linked to immune cell infiltration and activity. The objective of this study was to characterize the infiltrating cell types and immune profiles of tonsillar cancer in order to identify immunological mechanisms linked to HPV.

Biopsies from 17 patients with tonsillar cancer were collected. Paired contralateral healthy tonsil biopsies were collected from the same patients. The tumors were staged according to TNM classification and all biopsies were stained for p16 by immunohistochemistry, HPV typed, and quantified in terms of viral DNA load, transcriptional activity, and integration into the host’s genome by qPCR. All biopsies were immunoprofiled using 13-plex flow cytometry analysis of dissociated tissue and Nanostring nCounter®-based RNA analysis with the PanCancer Immune Profiling Panel encompassing 770 genes from 24 different immune cell types.

Of the 17 patients, HPV was found in 88% of the tonsillar cancer lesions and in 71% of the paired healthy tonsils with 93% being of HPV subtype 16 and 7% of HPV subtype 35. Flow cytometric analysis revealed a statistically significant increase in the frequencies of CD8+ T cells, CD13+ myeloid cells, and dendritic cells (DC), out of total CD45+ leukocytes, in tumor tissue compared to both healthy control tissue. In contrast, the frequencies of CD4+ T cells were decreased in tumor tissue as compared to healthy tissue, and CD11c+ conventional DCs were significantly less abundant in the myeloid population of the tumor, given their differential fold increase (6 fold and 10 fold respectively).

Differential expression profiles identified using Nanostring PanCancer Immune Profiling analysis of HPV+ and HPV- tonsillar cancer and healthy tonsillar tissue reveals patterns of ongoing or suppressed immune responses in these patient samples, which may serve as a foundation for selecting patient groups suited for immunotherapy.

Keywords: HPV, Dendritic cells, Tonsillar cancer, immuno-profiling.

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## B134 / Spatial heterogeneity of the T cell receptor repertoire reflects the mutational landscape in lung cancer

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Somatic mutations together with immunoediting drive extensive heterogeneity within primary lung tumors. Here, we examine tumor heterogeneity through the lens of the T cell receptor repertoire, which is fundamental in understanding the intricate relationship between the tumor and intratumoral T cell response. We evaluate TCR expansion and diversity across multi-region non-small cell lung cancer (NSCLC) samples, non-tumor adjacent lung tissue and peripheral blood, identifying a set of TCR sequences selectively expanded in tumor tissue. The number of expanded TCRs varies both within and between tumors, and correlates with the number of non-synonymous mutations within each tumor region. The expanded TCRs can be sub-divided into ubiquitous TCRs, found in every tumor region and regional TCRs, found in some regions but not others. The number of ubiquitous and regional TCRs correlates with the number of ubiquitous and regional non-synonymous mutations respectively. Expanded TCRs form part of clusters of TCRs of similar sequence, and with similar spatial distributions, suggestive of a spatially constrained antigen driven process. Expanded ubiquitous TCRs are a major fraction of PD1+CD57- CD8+ T cells bearing a classical tissue-resident and dysfunctional phenotype. Longitudinal analysis demonstrated that these ubiquitous TCRs are preferentially detected in the blood at the time of tumor resection, but not at subsequent follow-up. These findings highlight a non-invasive method to identify and track relevant tumor reactive TCRs for use in adoptive T cell immunotherapy.

Keywords: T cell receptor, Neoantigen, Lung Cancer, TRACERx.

## B135 / Recapitulating the orthotopic tumour microenvironment using bioluminescent syngeneic models in immune competent mice for checkpoint inhibitor interrogation

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Checkpoint inhibitors have shown promising therapeutic benefit in various cancer types, but more advanced preclinical models are required to combat resistance and evaluate opportunities for combination treatments. Subcutaneous syngeneic models are routinely used to evaluate the impact of immunotherapies on tumour growth as well as tumour invading leucocytes (TILs). However, the tumour microenvironment of orthotopic models is more comparable to the patient due to the organ-specific location of the tumour which facilitates metastatic spread and also recapitulates the immune and stromal component interactions with the tumour. Bioluminescent imaging (BLI) enables non-invasive longitudinal monitoring of orthotopic tumour burden and end stage quantification of tumour spread. Here we report the generation of a panel of bioluminescent syngeneic cell lines for orthotopic and metastatic modelling with an aim to assess the impact of standard of care agents, immune checkpoint therapies and combinations in a more clinically relevant environment.

Bioluminescent variants of syngeneic cell lines were established by lentiviral transduction. Orthotopic models were established via direct implantation into various organs e.g. liver, breast, pancreas and bladder and tumour growth assessed by BLI in-life as well as end stage (Spectrum CT; PerkinElmer). Response to standard of care agents such as sorafenib, gemcitabine, docetaxel and immune checkpoint therapy (anti-CTLA-4, anti-PD-1) was also evaluated and TIL infiltration was assessed by flow cytometry analysis and IHC. The success rate of tumour transplantation into different organs ranged from 60-100% as confirmed by both in-life imaging and ex vivo imaging at termination. For example, Hepa 1-6 appeared to grow within the parenchyma of the liver whereas 4T1 metastasized to the lungs and bone from the mammary fat pad, which was easily quantified by BLI. Real-time quantification of tumour size could be correlated with biomarkers and end stage assessment, for example, treatment with Sorafenib and check point inhibitors was correlated with alpha-fetoprotein (AFP) and end stage tumour burden, showing significant response in the orthotopic setting ($p<0.001$, Two way ANOVA). Different responses to check point inhibitors were observed between tumours inoculated at subcutaneous sites and orthotopic sites.

Bioluminescent syngeneic models enables clinically relevant interrogation of standard of care agents, immunotherapies and combination which is distinct from the subcutaneous setting. The orthotopic microenvironment influences the tumour growth and response to immuno therapy. These models also enable the assessment of disease progression and modelling the metastatic environment.

Keywords: Tumour microenvironment, Orthotopic, Bioluminescent, Checkpoint inhibitor.

## B136 / Expression profiles and function of IL6 and IL10 in tumor and myeloid cells

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Inflammation is a hallmark of tumor establishment, growth and progression. Cytokines are key mediators in the initiation and regulation of inflammation and can be broadly classified into 'pro' and 'anti' inflammatory roles. Key roles for the pro-inflammatory cytokine IL6, and the anti-inflammatory cytokine IL10 have been identified in colitis-associated colorectal cancer. However, the molecular mechanisms underlying their contribution to carcinogenesis remain unclear. Both IL6 and IL10 levels were found to be elevated at the transcriptional level in a mouse model of colitis-associated cancer, as well as in human colorectal cancer patients. Analysis of CD45- and CD45+ cells from the tumor microenvironment showed that while IL6 was expressed by both populations, IL10 expression was limited to the hematopoietic compartment. Intracellular staining and flow cytometry analysis of splenic and colonic lamina propria tissues revealed the cellular source of both IL6 and IL10 to be CD11b+Ly6CloLy6G+ immature myeloid cells. Addition of cultured supernatant from these cells to mouse colorectal cancer cell lines induced STAT3 phosphorylation, which mediated epigenetic remodeling of tumor cells through upregulating DNMT1 and DMNT3b expression, resulting in the silencing of the tumor suppressor IRF8. Blockade of IL6 signaling abrogated the STAT3-DMNT1/3b pathway. Similarly, treatment of colorectal cancer lines in vitro with recombinant IL6 or IL10 alone or in combination upregulated DNMT1/3b expression in a STAT3-dependent manner. However, dual treatment evidenced no additive or synergistic effect, suggesting that either IL6 or IL10 alone is sufficient to activate epigenetic remodeling. Deletion of IL6 in colorectal cancer cells, or use of an IL6-deficient host, failed to affect tumor growth kinetics in vivo. However, overexpression of IL6 significantly increased in vivo tumor growth, implying that a high threshold of tumor produced locally elevated IL6 exists for its previously reported growth-promoting effects. Our results suggest that targeting of inflammation-induced or tumor-associated CD11b+Ly6CloLy6G+ immature myeloid cells could abrogate both IL6 and IL10 production, inhibiting pro-tumorigenic epigenetic remodeling of colon epithelial cells.

Keywords: Colorectal Cancer, IL6, IL10, MDSC.

## B137 / Emergence and Behavior of Tumor-Resident Dendritic Cell Subsets during Anti-Tumor Immunity

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Checkpoint blockade therapies attain optimal responses in tumors infiltrated by T cells, limiting the number of patients who can benefit from this therapeutic approach. Because dendritic cells (DC) bridge the innate and adaptive immune responses, a better understanding of their roles in anti-tumor immunity can orient the development of novel immunotherapies. Recently, clinical and

preclinical studies have refocused attention to DC functions in the tumor microenvironment that follow initial priming, identifying CD103+ cross-presenting cDC1 as especially potent mediators of anti-tumor immunity. However, the emergence of tumor-resident DC subsets and their specific behaviors that contribute to anti-tumor immunity are poorly defined. These non-classical roles for conventional DC, and how they change over time, remain difficult to assess by bulk approaches that rely on surface markers to understand cell phenotypes in situ. Here we dissect the expansion and behaviors of tumor-resident vs migratory cDC using lineage tracing and intravital imaging. We asked whether conventional DC1 and DC2 are generated within the tumor microenvironment or are recruited from the periphery. Over several days, we tracked lineage-marked tumor-resident or migratory DC1 and DC2 within tumors and draining lymph nodes. Distinct populations emerged at both sites that were not mirrored by newly infiltrating DCs, suggesting the tumor microenvironment may profoundly dictate differentiation of DC subtypes in situ. We correlated these trends observed in bulk with behaviors of tumor-resident DC using intravital imaging over consecutive days. Quantitative image analysis of DC revealed cell morphological and cell motility shifts that corresponded with the generation of tumor-resident cDC1 and cDC2 subsets. Finally, we monitored DC trafficking out of tumors into the periphery in real time and over consecutive days by coupling an in vivo cytometry platform with photolabeling to mark tumor-resident DC. We quantified and captured tumor-experienced cDC shed into the periphery on consecutive days from the same mouse, demonstrating the feasibility of this approach for future studies. Our transplantation and imaging approaches reveal the origins and behaviors of DC residing in tumors and prompt novel avenues to perturb DC subsets, and thereby improve immunotherapies for cancer.

Keywords: Dendritic Cell, Migration, Microscopy, Lineage Tracing.

## B138 / Tumor-associated neutrophil subtypes in non-small cell lung cancer

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The aim of this study is to identify novel immune suppressive neutrophil subpopulations in the non-small cell lung cancer microenvironment (TME) and elucidate the mechanism of i) their recruitment and ii) the execution of their immune suppressive properties. Non-small cell lung cancer (NSCLC) represents ~85% of all lung cancer cases and although, surgical intervention can be curative for early stage patients, traditional chemo- and radiotherapy show limited effectiveness. Recent studies have demonstrated that negative regulators to T cell responses, such as immune checkpoint molecules (e.g. PD-1/PD-L1) and immune suppressive cell populations in the TME are likely to explain the failure of immune protection in many patients. Identifying the suppressive factors, cell entities and functions in the TME as well as the presence of an antigen-driven immune response is essential for the identification of a suitable immunotherapy. Agents targeting immune checkpoints (ICI therapy) seem to be remarkably effective in a wide range of tumors. Nevertheless, in NSCLC the overall response rates are ≤ 20%, making combinatorial protocols for patient subgroups highly needed. Several studies have pointed the potential importance of neutrophils in cancer. The neutrophil gene signature was found to predict mortality better than any other immune cell signature across 25 different cancers. Moreover, neutrophils were found to be the most prevalent immune cell type present in NSCLC and to inversely correlate with CD8+ and CD4+ content within tumor. These data suggest that neutrophils may act as immunosuppressive entity in the TME of NSCLC. In this study the following approaches have been followed: a) Fundamental immune response subtypes in NSCLC were identified by multi-parametric flow cytometry. Next, cellular drivers of these subtypes were determined by multiplexed immunohistochemistry and the CD8 / PMN ratio was used to predict ICI treatment outcome in NSCLC. b) Blood neutrophils from healthy individuals were incubated with human lung cancer cell line supernatants for functional assays such as shape change and chemotaxis, indicating the capability of cancer supernatants to induce an activated neutrophil phenotype. c) In order to identify novel tumor-associated neutrophil (TANs) subsets, we performed the profiling of over 300 human cell surface markers. So far, our analysis has indicated more than 30 markers with a significant differential expression between TANs and lung neutrophils. d) Finally, experiments in neutrophil – T-cell co-culture systems, revealed that neutrophils can bear lymphocyte suppressing properties. Overall, the goal of this study is to dissect neutrophil heterogeneity in the TME of NSCLC. Our data underlines the importance of the identification of TAN subpopulations with pro and anti-tumor properties which can offer a more specific target against immunosuppression and help the improvement of the overall response rates of NSCLC patients towards immune checkpoint inhibitor therapies.

Keywords: non-small cell lung cancer, tumor-associated neutrophils, TME analysis, tumor immunology.

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## B139 / The role of neutrophil-derived myeloperoxidase in non-small-cell lung cancer

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The aim of this study is to investigate whether myeloperoxidase (MPO), a neutrophil-derived molecule in the tumor microenvironment (TME), can influence tumor growth and T-cell function and thereby limit immune checkpoint inhibitor (ICI) therapies in non-small cell lung cancer (NSCLC). Understanding the role of neutrophils and MPO in ICI therapy may be a crucial step towards the potential clinical use of MPO inhibitors in lung cancer patients. Lung cancer, the leading cause of cancer-related deaths worldwide, is a heterogeneous disease comprising multiple histologic subtypes of which NSCLC accounts for ~ 80 %. Although immune-based therapies have shown initial promise in the treatment of lung cancer, just ~20% of NSCLC patients benefit from single agent ICI therapies and underlying mechanisms for treatment failure are mostly unknown. This highlight the need to identify additional molecules in the TME that limit ICI therapy. Our lab recently reported that neutrophils are the most prevalent immune cell type present in NSCLC and they constrain antigen-driven immune responses in tumor but not in non-adjacent lung tissue. Further, it has been shown that neutrophils in the TME can suppress lymphocyte proliferation and function and mouse studies revealed that neutrophils present in the TME limit ICI therapy however few is known about the mechanisms involved. In this context, some of the neutrophil cytoplasmic granule components have been proposed to contribute to tumor proliferation, angiogenesis and metastasis and moreover that they could regulate immune cell functions in the TME. One of these molecules is MPO,

a peroxidase enzyme that generates reactive oxygen/nitrogen species and is most abundantly expressed in neutrophils. Upon neutrophil activation, during acute inflammation or in the TME, MPO is secreted into the extracellular milieu during degranulation and utilizes H2O2 to hypochlorous acid that reacts with surrounding proteins and cell surface molecules and thereby can alter signaling pathways and cell functions. Using a genetic engineered mouse model, we found that MPO knockout mice exhibited prolonged survival when compared to the wildtype littermates and in the same model, CD8+ T-cells from the MPO knockout mice revealed altered activation/exhaustion markers expression. Furthermore, using a heterotopic syngeneic tumor model, we discovered that the MPO inhibitor 4-aminobenzoic acid hydrazide (ABAH) suppresses lung cancer promotion in mice since the ABAH-treated mice developed tumors later when compared with the vehicle group. Moreover, tumor growth rate and tumor volume were significantly reduced in the ABAH-received group. The comprehensively profile of the immune cell content on the primary flank tumors by flow cytometry revealed a shift in the percentage of lymphoid populations including T-cells as well as a shift in the composition and activation of CD4+ and CD8+ subsets when compared ABAH-treated mice vs controls. In vitro experiments using western blot and flow cytometry showed that MPO is able to bind and internalize in A549 lung adenocarcinoma cells. Finally, we observed that MPO treatment induces the phosphorylation of Akt and Erk in A549 cells which could be related with the regulation of cell proliferation and apoptosis. Our findings suggest that MPO may play a role in the development of lung cancer either by regulating cancer cells function or by influencing immune cells behavior and therefore immune-based therapies.

Keywords: Myeloperoxidase, Neutrophils, Non-small-cell lung cancer, Checkpoint inhibitors.

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## B140 / Site specific regulation of programmed death receptor 1 ligand in metastatic breast cancer reveals impact of lung tumor immune microenvironment

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For the first time in breast cancer, targeting programmed cell death receptor 1 pathway (PD-1/PD-L1) demonstrated substantial benefit on overall survival in a minority of immune cell PD-L1 positive, metastatic triple-negative breast cancer patients (TNBC). Nevertheless, the vast majority of patients is not responding to immune checkpoint blockade due to primary resistance and insufficient pre-existing T-cell immunity.

We believe that immune evasion is orchestrated by various, coincident mechanisms depending on time as well as site specificity. Consequently, our objective was to identify patterns of immune responses during the transition of primary to metastatic breast cancer and to explore the tumor immune microenvironment comparing different organ sites. Two independent, matched cohorts of 67 (various organ sites) and 58 (lung) breast cancer patients were analyzed by in depth immunohistochemistry and mRNA expression levels (RT-qPCR) of key regulatory immune related genes and clinical as well as histopathologic parameters.

First, we observed a less immune related activity by temporal downregulation of stromal tumor infiltrating lymphocytes (TILs, p < 0, 0001), CD8 mRNA (p < 0, 0001) and PD-L1 mRNA (p < 0, 0059). Other important targets such as CD68, TIM-3 and LAG-3 were preserved in metastatic disease. On the patient level, using 4 categories of immune response types applying CD8 and PD-L1 (Ignorance/Resistance/Tolerance/Induction) immunological ignorance was most frequent (48/67). Nevertheless, a temporal switch of immune response types in all directions became apparent. Moreover, unsupervised hierarchical clustering identified prognostic relevant signatures depending on CD8, PD-L1 and interferon-γ signalling (IFNG) with an impaired distant disease free (DFS) and overall survival (OS) for CD8+PD-L1+IFNGlow patients. However, significant differences depending on the metastatic organ site has been found for protein and mRNA levels of PD-L1 on tumor and immune cells in the lung, compared to other tissues (bone, liver, soft tissue, brain; p=0, 0033). In contrast, only patients with pulmonary metastasis (n=58) revealed a Th1 driven microenvironment in a majority of samples associated with preserved immune cell PD-L1 in pulmonary metastasis compared to primary tissue. This was corroborated by numerically increased infiltration of stromal TILs.

In conclusion, our findings indicate a switch to a less immunoreactive environment in metastatic compared to matched, primary samples of breast cancer patients. Further, we demonstrate induction of T-cell mediated immunity across all biologic subtypes, more frequent associated with pulmonary tumor microenvironment including considerably high expression of immune cell PD-L1 which may be predictive in the clinic.

Keywords: lung metastasis, breast cancer, PD-1, PD-L1.

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## B141 / Immune regulatory effects of tumor associated natural killer cells in glioma

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NK cells are commonly seen as the first line of innate anti-tumor immune response and as such strongly shape the tumor environment. However, in analogy to T cells, tumor associated NK cells (TANKs) can adopt a dysfunctional and exhausted phenotype in late stage tumors. Recent studies have shown a prominent immune-regulatory role of NK cells especially in chronic viral infections. Furthermore, NK cells have already been shown to develop a regulatory phenotype in experimental auto-immune disease by direct killing of activated antigen-specific T cells. Few studies have found indications that suggest a similar role for NK cells in a tumor setting. Nonetheless, the phenotypical shift and underlying mechanisms remain unclear.

We found a substantial number of infiltrating NK cells in human low- and high-grade gliomas as well as experimental mouse gliomas. Using single-sample GSEA of the glioblastoma TCGA dataset we found that the abundance of cells matching the NK cell signature correlated with poorer survival. Analysis of a comprehensive preclinical dataset generated in our lab revealed that NK cells are enriched in late stage tumors of mice non-responsive to immune checkpoint blockade. In co-culture experiments, NK cells sorted from intracranial tumors suppressed T cell proliferation whereas circulating NK cells had the opposite effect. Depletion of NK cells also led to decreased tumor growth and inhibited infiltration of immunosuppressive peripheral macrophages in a murine glioma model. Further investigations will aim to phenotypically characterize NK cells and their shift towards a regulatory phenotype using single cell transcriptomics. Collectively, these data suggest that glioma-infiltrating NK cells represent a previously unrecognized important component of the immunosuppressive glioma microenvironment and a potential therapeutic target.

Keywords: Natural killer cells, Immune regulation, Glioma, Glioblastoma.

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## B142 / Alterations of the bidirectional cross-talk between pDCs and γδT cells in circulation and within the tumor microenvironnement of melanoma patients

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Melanoma escape from anti-tumor immune control is not yet fully elucidated. Understanding the mechanisms of this subversion is crucial to optimize ongoing immunotherapies and design new therapeutic strategies to achieve better clinical success. Dendritic cells (DCs) and anti-tumor effectors are crucial in cancer immunity. We previously demonstrated that melanoma hijacked both plasmacytoid DCs (pDCs) and γδT cells to escape from immune control. Indeed, tumor-infiltrating pDCs are associated with a poor clinical prognosis and their modulation within the tumor micro-environment leads to the subversion of T cells toward regulatory T and TH2 responses. Besides, circulating and tumor-infiltrating γδT cells from melanoma patients display an altered expression of Natural cytotoxic receptors (NCR), Killer-cell immunoglobulin-like receptors (KIR) and immune checkpoints, and, in response to stimulation with phosphoantigens, had an impaired ability to express activation molecules, secrete cytokines and display cytotoxicity toward tumor cells. We investigated here the impact of pDCs and γδT cells deregulations on their cross-talk in the context of melanoma. pDCs and γδT cells were purified from healthy donor (HD) (blood) and melanoma patients (blood and tumor) and cocultured together. We studied the modifications of γδT-cell features induced by pDCs in presence or not of TLR7/9-L or zoledronate, and looked for modulations of pDC features triggered by γδT cells in presence or not of phosphoantigens. We show that circulating and tumor-infiltrating pDCs from patients were defective in activating healthy γδT cells and eliciting their cytotoxic activities, and triggered an increase in IL-4/10/13/17 cytokines associated with a lower production of IFNγ and TNFα compared to pDCs from HD. Furthermore, circulating and tumor-infiltrating γδT cells from patients were defective in triggering pDC activation, and failed to induce IFNα/IP10 production and TRAIL expression compared to γδT cells from HD. We also identified the molecular mechanisms governing pDC/γδT-cell cross-talks, which involve both soluble factors and membrane contacts. Thus, we demonstrate here that pDCs and γδT cells cross-regulate each other, these interactions being altered in a melanoma context. Such understanding of the physiopathology of pDCs and γδT cells may help designing new therapeutic approaches exploiting their antitumor potential while counteracting their skewing by tumors to improve patient outcomes.

Keywords: immunosubversion, gamma delta T cells, plasmacytoid dendritic cells, Melanoma.

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Girard et al., Oncoimmunol, 2019

## B143 / Role of endothelial CCL2 and CXCL10 for tumor immunity in the central nervous system

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A major challenge in glioma immunotherapy is to ensure homing of peripherally activated adaptive immune cells to the tumor while retaining their effector function. Preliminary work has shown synergistic effects of T cell-based immunotherapy and low-dose tumor irradiation in a syngeneic murine glioma model and suggests a major role of vasculature-mediated mechanisms for T cell transmigration and activity. This project aims to identify and prove evidence of specific endothelial factors mediating immune response within the glioma vasculature after local irradiation, thus delineating their role in controlling homing and activity of glioma-infiltrating T cells.

Using a syngeneic murine GL261 glioma model expressing the major histocompatibility complex (MHC) class I-restricted model antigen glycoprotein-100 (gp100), we could show that local glioma irradiation enhances the efficacy of T-cell based immunotherapy. In vivo two-photon microscopy and correlative ultramicroscopy of ex vivo cleared whole brains demonstrated co-localization of fluorescently labeled tumor blood vessels with adoptively transferred T cells and an increased T cell transmigration after irradiation, which points to the vascular compartment as key mediator of radiation-induced anti-tumor immune response.

Gene expression profiles of tumor blood vessels of primary and recurrent human glioblastoma samples provide a first evidence of an angiocrine effect on tumor immune microenvironment. In vitro, human brain endothelial cells showed increased expression of CCL2 and CXCL10 after irradiation. Furthermore, immunofluorescent co-staining on murine glioma tissue indicated co-localization of CXCL10 and CD31 after adoptive T cell transfer and irradiation. These results suggest an important role of chemokines like CCL2 and CXCL10 for irradiation-mediated recruitment of macrophages and antigen-specific T cells to gliomas.

The precise mechanisms behind the spatiotemporal angiocrine

regulation of tumor immunity and the immune-vascular cross-talk within the central nervous system need to be validated for a deeper understanding of the vascular control and could provide a key therapeutic target to enhance anti-tumor immune response.

Keywords: glioma irradiation therapy, T cell transmigration, immune-vascular cross talk, tumor microenvironment.

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## B144 / Mutational burden is associated with compartment-wide features of intratumor CD4 T cell dysregulation in lung cancer

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Background: Persistent antigen exposure has a detrimental effect on immune function, associated with CD4 T cell differentiation skewing (CD4ds) resulting from decline of an early differentiated population in favour of later differentiated, dysfunctional subsets. Whilst this has been described in chronic viral infections and autoimmunity, it is unknown whether CD4ds occurs within the tumour microenvironment contributing to immune failure to control tumour growth.

Methods: We combined tumour infiltrating lymphocyte (TIL) high dimensional flow cytometry, bulk exome and RNA sequencing data from multiregional samples obtained from surgically resected tumours of treatment naive patients with non-small cell lung cancer (NSCLC) amongst the first 100 recruited to the prospective, UK-wide lung TRACERx study. We additionally analysed publically available single T cell RNA sequencing data and bulk RNA sequencing data within TCGA.

Results: We found CD4ds to occur amongst TILs in association with tumour mutational burden (TMB). Loss of PD1-CCR7+ T central memory enriched early differentiated cells was accompanied by gain in abundance of PD1+ populations with exhausted (CD57-ICOShiCTLA4hi) and terminally differentiated effector (CD57+Eomes+) features. Identification of these subsets within single T cell RNA sequencing data revealed shared and distinct functional regulators. We validated a transcriptional signature of CD4ds using TRACERx samples with paired flow cytometry and RNA sequencing data. This signature was found to predict worse survival within TRACERx and multiple TCGA cohorts including lung adenocarcinoma (LUAD). As TMB was found to be an independent predictor of good outcome in multivariable survival analysis, we reasoned factors other than mutational burden could promote CD4ds. Regulatory T cell (Treg) abundance was found to inversely correlate with infiltrating early differentiated cells, independent of mutational burden. Analysis of bulk and single cell RNA sequencing revealed Treg expression of CCR3 and CCR8 as potentially key factors regulating the intratumoural abundance of this subset.

Conclusion: Remodelling of the CD4 differentiation landscape occurs in association with tumour mutational burden, suggesting that chronic stimulation by tumour antigens may lead to loss of immune fitness through depletion of early progenitors and gain in abundance of dysfunctional subsets. Tregs may additionally exert an influence in this process. Our analysis of transcriptomic data elucidates potential regulatory mechanisms and therapeutic targets within the CD4 subsets identified.

Keywords: Lung cancer, CD4 differentiation, Mutational landscape.

## B145 / TIM-3 dictates functional orientation of the immune infiltrate in ovarian cancer

Jitka Fucikova (Sotio).

Purpose: In multiple oncological settings, expression of the co-inhibitory ligand PD-L1 by malignant cells and tumor infiltration by immune cells expressing co-inhibitory receptors such as PD-1, CTLA4, LAG-3 or TIM-3 conveys prognostic or predictive information. Conversely, the impact of these features of the tumor microenvironment on disease outcome amongst high-grade serous carcinoma (HGSC) patients remains controversial.

Experimental Design: We harnessed a retrospective cohort of 80 chemotherapy-naïve HGSC patients to investigate PD-L1 expression and tumor infiltration by CD8+ T cells, CD20+ B cells, DC-LAMP+ dendritic cells as well as by PD-1+, CTLA4+, LAG-3+ and TIM-3+ cells in relation with prognosis and function orientation of the tumor microenvironment. Immunohistochemical data were complemented with transcriptomic and functional studies on a second prospective cohort of freshly resected HGSC samples. In silico analysis of publicly available RNA expression data from 308 HGSC samples was used as a confirmatory approach.

Results: High levels of PD-L1 and high densities of PD-1+ cells in the microenvironment of HGSCs were strongly associated with an immune contexture characterized by a robust TH1 polarization and cytotoxic orientation that enabled superior clinical benefits. Moreover, PD-1+TIM-3+CD8+ T cells presented all features of functional exhaustion and correlated with poor disease outcome. However, while PD-L1 levels and tumor infiltration by TIM-3+ cells improved patient stratification based on the intratumoral abundance of CD8+ T cells, the amount of PD-1+ cells failed to do so.

Conclusion: Our data indicate that PD-L1 and TIM-3 constitute prognostically relevant biomarkers of active and suppressed immune responses against HGSC, respectively.

Keywords: TIM-3, immune check-points, tumor microenvironment, ovarian cancer.

References:
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## B146 / Canonical and non canonical functions of C1q in cancer progression

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The complement system plays an important role on tumor progression by acting on the different key features of the tumor; fundamental functions of tumor cells, angiogenesis and modulation of the immune infiltrate. However, this effect seems to be cancer type dependent and this has to be taken into account in the establishment of potential biomarkers and development of therapeutic strategies.

By bioinformatic studies, we have characterized the intratumoral complement landscape in more than 10, 000 tumors comprising 33 tumor types by utilizing data compiled by TCGA. This pan-cancer analyse of complement reveals a high heterogeneity of complement gene expression and prognostic value of complement

genes between cancer types. Across cancer types, we identified 2 major subtypes according to prognostic significance of classical/alternative pathway genes– Agressive complement tumors and protective complement tumors.

Clear cell renal cell carcinoma (ccRCC) highly expressed complement genes and belongs to the agressive complement tumors. In vitro, we have shown that the classical pathway of complement system can be activated locally through on dynamic interaction between macrophages that produce C1q and tumor cells which produce the others proteins of the complement cascade (C1r, C1s, C2, C4, C3). The initiation of the classical pathway takes place after the fixation of the C1 complex to IgG deposits on tumor cell membrane. In 3 independent cohorts of ccRCC patients we found that the presence of C1q producing cells is associated with bad prognosis.

In under to better understand if the pro-tumoral effect of C1q is linked to the activation of the cascade (canonical functions) or not (non canonical functions), we have setted up different mouse models deficient for C1q or C4 in which we have injected different cancer cell lines (TC1, MC38, MCA205). The analyses of the different key features of the tumors – immune infiltrate, angiogenesis and proliferation – reveal that some effects are linked to the canonical functions of C1q (modulation of the immune environment) but others are independent of the cascade (angiogenesis). The modulation of the immune system by C1q towards an exhaustive phenotype, lead us to investigate new therapeutic strategies by combining anti PD1 treatment and C1q deficit in mice.

Keywords: complement, classical pathway, clear cell renal cell carcinoma.

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Roumenina*, Daugan*. et al. Tumor cells hijack macrophage-produced complement C1q to promote tumor growth. Cancer Immunol. Res. 2019

## B147 / Investigating the status of the tumor microenvironment in Li-Fraumeni Syndrome

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Li-Fraumeni Syndrome (LFS) is a highly penetrant cancer predisposition syndrome associated with germline mutations of the TP53 tumor suppressor. LFS patients face a significantly elevated lifetime risk of developing a broad spectrum of early-onset malignancies including sarcomas, breast and brain cancers. Emerging evidence has implicated p53 as a mediator of immune responses. Of particular relevance for anti-tumor immunity are proposed roles for p53 in the regulation of immune checkpoints and natural killer (NK) cell activation. However, the immunological status of LFS patients, in whom p53 function is reduced or altered, is unclear. Given the prognostic significance of intra-tumoral immune infiltration and interest in cancer immunotherapy, including immune checkpoint inhibition, as a treatment modality for clinically challenging malignancies such as those characteristic of LFS, study of the immunological status of these patients is imperative. The objective of our study was to to clarify how p53-dependent processes in immune cells might regulate the composition of the tumor microenvironment in LFS-associated cancers. We analyzed 51 unique tumors of various histological types procured from pediatric LFS and non-LFS cancer patients. These tumors were subjected to bulk RNA-sequencing and various immunogenomic analyses - namely, immune contexture deconvolution with the quanTIseq and EPIC modules and single sample gene-set enrichment analysis with the xCell and MCPcounter modules, to qualify and quantify the tumoral immune cell composition. Additionally, blood-derived DNA methylation analysis was performed on 134 samples procured from LFS patients either affected or not affected with various LFS spectrum cancers. Differential expression of key immune-related gene promoter regions was observed in LFS patients affected versus unaffected with cancer. Specifically, FOXP3, CTLA4, and ICOS, were hypomethylated in LFS patients affected with cancer, and ULBP1 was hypermethylated in these patients. Additionally, analysis of methylation-based immune contexture deconvolution revealed NK cells to be associated with cancer status in LFS patients – LFS patients affected with cancer had a lower normalized fraction of NK cells versus those who were unaffected. Preliminary transcriptomics-based immunogenomic analyses have suggested that certain LFS-associated tumors may have impaired anti-tumor immunity, possibly associating with the prognosis of the tumor type in question. NK cells, CD8+ T cells, and endothelial cells were significantly depleted in LFS patient tumors relative to their quantified proportions in sporadic pediatric tumors of related histological types. Further characterization of p53-dependent processes in immune cells and their relevance in LFS could help to inform future study and use of immunotherapeutic agents in this clinical context with the ultimate goal of improving the clinical management and survival of LFS patients with cancer. This project is the first comprehensive analysis of the immunological status of LFS and utilizes the largest LFS cohort to-date. Our findings illustrate the contribution of genetic changes in LFS to the composition of the tumour immune microenvironment and highlight how these may be associated with cancer status.

Keywords: Li-Fraumeni Syndrome, Immunogenomics, Tumour Microenvironment, Computational Immunology.

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## B148 / All-in-one flow cytometry staining panel for immune-cell profiling in syngeneic tumor models

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Checkpoint inhibitor treatment has become a common therapy of various cancer types; however, clinical data indicate that only few patients respond to this regiment due to attenuated anti-tumor immune response. Thus, it has been recognized as important to consider the immune response already during preclinical drug development to anticipate such clinical drawbacks and investigate the changes of immune cell populations after treatment. Flow cytometry is widely used for this kind of analysis. However, a comprehensive analysis of numerousness immune cell populations in one tumor is a major challenge when only limited material is available due to small tumor size of e.g. a tumor responding to the treatment or analysis at an early tumor developmental stage. Having a staining panel that allows the analysis of all major immune cell populations in a single staining would be a major advantage.

On Day 0, syngeneic CT26wt colon tumor cells were implanted subcutaneously (s.c.), into the mammary fat pad (i.ma.) and orthotopically (ortho), respectively. On Day 4 after implantation, the tumor bearing mice were randomized in two groups and treatment started. Group 1 was treated with vehicle only, whereas Group 2 received anti-mCTLA-4 antibodies every 3rd day. All CT26wt models responded well to immune-checkpoint inhibitor therapy. On the day of randomization (Day 4), Day 7, Day 10 and Day 13 after implantation five animals were euthanized and the tumor harvested for flow cytometry analysis. The single cells were stained for live/dead and the antigens CD3, CD4, CD8a, CD45, CD25, CD11b, Ly6C, Ly6G, F4/80, CD11c, MHC class II, CD206, CD335, CD49b, B220 and FoxP3 and analyzed by flow cytometry. With a superior gating strategy, the all-in-one staining panel yielded more accurate results than previously obtained with less

markers. By this single staining also little tumor material was analyzed for all major immune cell populations. The selected early time points during tumor development revealed strong dynamic changes in the immune populations throughout all models. These changes in the vehicle and the effect by the anti-mCTLA-4 treatment are presented.

Keywords: flow cytometry, CT26wt, immune cell populations, comprehensive analysis.

## B149 / Exome-capture RNA sequencing of laser-capture microdissected cancer cells and immune cells from formalin-fixed paraffin embedded breast cancers

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Tumors are conserved in clinical pathology as formalin-fixed paraffin-embedded (FFPE) blocs. Formalin fixation results in chemical alteration of nucleic acids rendering transcriptomic analysis challenging. Moreover, RNA sequencing is usually performed on tumor bulk, without distinction of diverse cell types. Here we describe the development of a robust method of RNA extraction and RNA sequencing of laser-capture microdissected tumor cells and immune cells from aged (1-10 years) FFPE blocks (tumor specimens and microbiopsies) of 7 samples of triple negative breast cancers (TNBC). Cancer cells and immune cells were microdissected separately based on their morphology. Exome-capture RNA sequencing was performed to characterize cell types separately. Unsupervised clustering and principal component analysis showed a clear separation between the transcriptome of microdissected tumor cells and immune cells. Immune cells were enriched in markers of B cells (PAX5, CD19, CD79A, FCMR and CD22) and strongly express adenosine receptor ADORA2A, a checkpoint that inhibits innate and adaptive immune response. Together, this study showed the feasibility of laser-capture microdissection and exome-capture RNA sequencing from FFPE breast cancers to better characterize tumor microenvironment and identify new targets for immunotherapy.

Keywords: RNA sequencing, Laser-capter microdissection, Tumor microenvironment, Triple negative breast cancer.

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## B150 / Quantifying tumor heterogeneity and mapping complex immune cell interactions with high-throughput, 7-color multispectral slide scans

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Background We describe two advances in multispectral fluorescence immunohistochemistry, a powerful tool for quantifying interactions within the tumor microenvironment. First, a fully-automated 8-plex assay plus DAPI counterstain on the same tissue section. Second, a novel scanning method that produces a multispectral whole slide scan of 6 markers plus DAPI counterstain in ~6 minutes (1x1.5 cm tissue section).

Methods FFPE primary tumors were immunostained using Opal™ reagents manually or on a Leica BOND RX™. Imagery was acquired on a Vectra Polaris® automated imaging system and analyzed with inForm® and MATLAB® software.

results A combination of 8 Opal™ dye reagents, from Akoya Biosciences Opal 9-color kits, were used to stain and distinguish 8 markers plus DAPI when imaged on the Vectra Polaris®. Figure 1 shows a 9-color panel on lung cancer: CD20 (Opal 480), PD-L1 (Opal 520), CD8 (Opal 540), FoxP3 (Opal 570), CD68 (Opal 620), PD-1 (Opal 650), Ki67 (Opal 690), and PanCK (Opal 780). Colors assigned to each marker, and associated component planes, are shown in Figure 1B.

These 8 markers combine to generate more than 20 phenotypes relevant to immuno-oncology that can be studied in relation to local PD-L1 expression and proliferation state (Ki67+/-). For example, while the density of CD8+ cells was 8-fold lower in tumor than stroma (150 vs 1200 cells/mm^2), those CD8+ cells were >4x more likely to be proliferating in tumor vs stroma (28% vs. 6%).

To interrogate interactions across a whole section, we additionally developed a multispectral whole-slide scanning method, demonstrated on lung cancer using a subset of 7 stains from the 9-color panel above. Phenotype and expression-level assessments of the unmixed whole slide scan describe distribution patterns of immune cells across the entire section.

In measurements of crosstalk and dynamic range, whole-slide multispectral scanning performed comparably to established field-based multispectral imaging, and outperformed conventional fluorescence scanning by reducing crosstalk from up to 8% to under 2% (typically <0.5%) and extending the dynamic range of some channels by more than 50-fold.

Conclusion We introduce a 9-color fIHC assay that distinguishes 8 markers plus DAPI counterstain on the same tissue section, increasing the depth of cellular interactions that can be studied within the tumor microenvironment. Additionally, we introduce a whole slide multispectral imaging method that provides rich quantitation of interactions among 6 markers at length scales spanning from cell biology to tumor physiology.

Keywords: Multiplexed Immunostaining in situ, Tumor infiltrating lymphocytes (TILs), Spatial distribution, biomarkers.

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## B151 / Impact of a-Radioimmunotherapy on tumor microenvironment

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Actual cancer therapies are facing numerous challenges towards tumor cell destruction: hypoxia is a limit to optimal efficacy of external irradiation, and poor tumor vascularisation restrains access to tumor cells for treatment like Immunotherapies. Furthermore, tumor microenvironment involves immunoregulatory cells and cytokines, which prevent anti-tumoral immune response. Therapeutic combination could be the key to turn these« cold» tumor microenvironment into« hot» ones: more vascularised and infiltrated with immune cells. To this end, this project focus on combining alpha-Radioimmunotherapy (a-RIT) and adoptive transfer of T-cells (ACT).

This therapeutic combination was conducted in a Multiple Myeloma (MM) murine model using a 5T33-OVA MM cell line expressing the CD138 antigen and H2Kb/OVA257-264 complexes grafted subcutaneously to C57BL6/KalwRij mice.a-RIT was delivered through i.v. injection of a radiolabelled antibody targeting CD138 allowing local irradiation of the tumor. To further reinforce its efficiency, a-RIT was combined with an ACT of tumor specific OT-1 T-cells. This therapeutic combination resulted in a significant tumor growth delay and improved survival, compared to ACT or a-RIT alone (Ménager and al, 2015). Based on these results, the aim of this project was then to understand the impact of a-RIT on the "cold" tumor microenvironment and on ACT efficacy. Tumor infiltrated cells were analysed by flow cytometry to identify in situ immune populations and their role in tumor growth inhibition. A significant decrease of CD8 and regulatory CD4 T cells was induced in the tumor microenvironment after a-RIT. After ACT, infiltration of OT-1 T-cells was observed in the tumor, to a larger extend when tumor had been previously treated with a-RIT. The motility of these OT-1 T-cells, studied by video-microscopy ex vivo, was also increased in tumors treated with a-RIT.

Combining a-RIT and ACT appears to turn this "cold" tumor model into a "hot" one by depleting tumor cells and regulatory T cells on the one hand and by increasing the proportion and motility of tumor-specific CD8 T cells on the other hand. Next, we aim to investigate impact of this combination on metabolism, hypoxia and secretion of chemokines in tumor microenvironment.

Keywords: radioimmunotherapy, adoptive cell transfer.

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## B152 / Deep proteomic profiling of CD4+ and CD8+ T cell-depleted tumor models in PD-1 treatment

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Background Immunotherapies targeting the PD-1/PD-L1 axis have been shown to be effective in only ~20% of cancer patients and the mechanism of action (MOA) underlying the differences between responders and non-responders remains poorly understood. Immunophenotyping of baseline samples (without treatment) play important roles in understanding MOAs, however a clear understanding of the heterogenous responses remains elusive due to the intrinsic complexity of tumor immunity. It is therefore critical to understand the roles of different lineages of immune cells in mediating PD-1 response, while gaining an overall understanding of the interplay between tumor microenvironment (TME) and immune cell populations. To address this, tumor-bearing syngeneic mice were treated with an anti-PD-1 antibody in combination with targeted CD4+ or CD8+ T cell depletion with the purpose of understanding how these lineages impacted anti-PD-1 efficacy. Tumor tissues were subsequently investigated with an unbiased proteomics workflow based on data-independent acquisition (DIA/SWATH) mass spectrometry to provide insights into TME in different treatment contexts.

Methods T cell subpopulations were depleted in two subcutaneous murine syngeneic models (MC38, and Hepa 1-6) followed by anti-PD-1 treatment (10mg/kg). The depletion of CD4+ and CD8+ T cells were accomplished with anti-CD4 (GK1.5) and anti-CD8 (2.43) antibodies. The effectiveness of each depletion was assessed by tumor growth, followed by flow cytometry analysis of tumor infiltrating lymphocytes. FFPE tumor tissue samples, taken at the end of study (17-20 days), were prepared for mass spectrometry using standard procedures. All samples were analyzed using 4h gradients on a C18 column coupled to a Thermo Scientific Q Exactive HF-X mass spectrometer. Data was extracted using Spectronaut™ (Biognosys) with sample specific and resource spectral libraries. Statistical analysis was conducted to identify candidate proteins that differentially expressed across treatment groups.

results Consistent with expectation, depletion of CD8+ T cells significantly attenuated the antitumor effects of anti-PD-1 treatment in both tumor models, confirming the crucial roles of CD8+ T cells in tumor cell killing and inhibition of growth. However, depletion of CD4+ T cells showed opposing effects in the two models tested with a significant enhancement of anti-PD-1 efficacy in MC38 tumors and a reduction of anti-PD-1 efficacy in Hepa1-6 tumors. In our proteomics analysis, a protein inventory was created and combined with resource assays to cover over 10'000 unique pro-

teins (peptide and protein false discovery rate <1%). DIA quantification runs will be performed and univariate statistical testing (q-value > 0.05 and log2 fold change > 0.58) will identify proteins dysregulated in the tumor tissue. Multivariate (PLS-DA) analysis will also identify candidates that are most strongly dysregulated between treatment subgroups of MC38 and Hepa1-6. Pathway analysis highlights differentially regulated biological functions and predicts upstream regulatory pathways.

Conclusion This study shows an unequivocal role of CD8+ T cells in anti-PD-1 induced tumor growth inhibition. However, the role of CD4+ T cells is different depending on the specific tumor type and TME. Here we apply proteomic profiling of FFPE tissues using DIA-MS enables to attempt to understand the interdependency of immune cells and the TME.

Keywords: Immuno-Oncology, Proteomics, T cells, Tumour microenvironment.

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Effect of immune cell depletion on response to PD-1 treatment in syngeneic models. Ying Jin, Yongli Shan, Chunkai, Li, Annie X. An, Henry Q. Li, Davy X. Ouyang. AACR annual meeting 2019, Poster Number 1503

## B154 / Using Quantitative Systems Pharmacology modeling to predict response and resistance of immune checkpoint inhibitors (ICI) in murine syngeneic tumors

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Objectives: Experimental mouse syngeneic models are commonly used to explore mechanisms of action for immunotherapies, as well identification of potential immune biomarkers correlating with tumor response or resistance. Syngeneic models also differ from one another, in their susceptibility to immunotherapy treatment and baseline immune conditions. The objectives of this work: (1) to develop a quantitative systems pharmacology (QSP) model, which describes the dynamics of tumor growth during immune checkpoint inhibition (ICI, anti PD-1, anti CTLA-4 or their combination) across various syngeneic models; (2) to link parameters of the model with baseline immune biomarkers; and (3) to validate the model via prediction of independent experimental data.

Methods: The mechanistic, population QSP model is based on our previous work [Mosely, S. et al., 2017]. It was used to incorporate data from six syngeneic tumors (4T1, LLC, CT-26, MC-38, B16, RENCA) into one quantitative framework, by capturing corresponding differences in tumor microenvironment (TME) baseline conditions as well as immune cell – tumor cell interactions [Kosinsky Y. et al, 2018] under anti PD-L1 and CTLA-4 mAb therapies in these six syngeneic tumors. Variability in individual tumor size dynamics was taken into account using a mixed-effects feature in the model, at the level of tumor-infiltrating T cell influx.

Results: The model adequately described individual- and cohort-level tumor size dynamics patterns, for all treatment regimens across all six syngeneic tumors. Anti PD-L1 therapy was incorporated into the model via a direct increase in an immune activation rate (IAR) function within the TME, thereby confirming the validity of our previous results [Kosinsky Y. et al, 2018]. Also, the QSP model adequately described observed differences in treatment responses, depending on the start of treatment time (i.e., tumor age). In the frame work of external cross validation, the developed QSP model accurately predicted the absence of treatment response in Pan-02, based solely on TME baseline conditions and pre-wired immune cell – tumor cell kinetic interactions in the model. Using the model, we hypothesised that the main immunosuppressive factor in Pan-02 could be traced to the high density of immunosuppressive myeloid cells in the TME, which can explain intrinsic resistance of this syngeneic model to ICIs [Kaneda M. et al, 2016]; (2) In A20 tumors, on the contrary, the QSP model predicted a 40% to 80% of animals with complete tumour rejection during ICI treatment, in excellent agreement with published data. Using the model further, we inferred that response to anti CTLA-4 therapy was associated with relatively high Treg numbers at baseline, in A20 TME [Pachter A. et al, 2017].

Conclusion: The mechanistic QSP model demonstrated its suitability for predicting tumor size dynamics in response to various ICI treatments in several syngeneic models. In particular, it was shown that one of the key factors influencing the treatment efficacy (or resistance) under PD-L1, CTLA-4, or combination ICI in syngeneic models, is the relative abundance of regulatory T cells and immunosuppressive myeloid cells.

Keywords: systems pharmacology, immune profiling, syngeneic models, dug combinations.

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## B155 / Modeling the tumor immune microenvironment of melanoma brain metastases

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Melanoma is the deadliest skin cancer and the American Cancer Society estimates that it will cause 7230 deaths in 2019. Up to 50% of these deaths will be due to brain metastasis. Recently immunotherapy with checkpoint inhibitors has emerged as a promising new avenue of treatment. Clinical trials of CTLA4 and PD1 blockade have resulted in increased survival of stage IV melanoma patients, with some patients experiencing shrinkage of brain metastases. However, responses are highly variable, and it is not completely understood why some patients respond better than others. In order to develop more effective immunotherapy strategies, a thorough understanding of how the immune system interacts with melanoma in the brain is needed. Our laboratory has established a model of immune-mediated tumor regression using YUMMER1.7, an immunogenic murine melanoma cell line driven by mutations central to human melanoma formation. When YUMMER1.7 is injected subcutaneously into immunocompetent mice, tumors are rejected spontaneously. However, upon intracardiac injection, YUMMER1.7 forms progressive brain metastases, even when injected at doses as low as 5000 cells per mouse. Surprisingly, YUMMER1.7 brain metastases exhibit microscopic fragmentation and cell death, reminiscent of the rejection response in the skin. This suggests that although it is not robust enough to cause rejection, the brain can mount an immune response. Furthermore, this immune response is dependent on adaptive immunity, as Rag-/- mice lacking functional T and B cells exhibit significantly decreased survival compared to WT mice. Preliminary characterization of the local microenvironment reveals colonization of the tumor site by local stroma, as well as the presence of CD45+ cells, indicating that immune infiltrate can penetrate into brain metastases. Finally, treatment of YUMMER1.7 brain metastases with anti-CTLA4 and anti-PD1 antibodies results in robust tumor regression and sustained survival, consistent with the survival rates observed in clinical trials. In summary, we have developed a model of melanoma brain metastasis that results in tractable, progressive tumors in an immunocompetent context and that respond to immunotherapy. Future work will focus on further characterization of the immune pathways modulating YUMMER1.7 growth in the brain, as well as determination of the specific mechanisms of immune response after checkpoint inhibitor treatment. Overall, this study will elucidate how the tumor immune microenvironment affects metastatic melanoma growth in the brain, allowing for the development of more effective, rationally designed immunotherapies.

Keywords: Melanoma, Metastasis, Brain, Immune microenvironment.

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## B156 / Highly multiplexed single cell spatial analysis of the tumor microenvironment in lymphoma

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Cancer immunotherapy, and checkpoint inhibition in particular, is poised to radically transform our approach to treating cancer. Recent clinical successes with checkpoint inhibitors and immuno-oncology agents have demonstrated the importance of the immune system in controlling and treating cancers. Early indications that tumor cells can generate an antitumor immune response were evidenced by the varying degrees of immune cell infiltration found in the tumor immune microenvironment (TME). Despite the presence of this immune infiltrate, is has long been suspected that immune function is defective or inhibited by the TME. Lymphomas are divided into Hodgkin and non-Hodgkin lymphoma, with diffuse large B cell lymphoma (DLBCL) being the most subtype of non-Hodgkin lymphoma. Both types of lymphoma demonstrate expression of PDL-1 on tumor cells but they have very different responses to treatment with PD1/PD-L1 targeting agents. For example, two recent clinical trials with nivolumab demonstrated response rates in Hodgkin lymphoma of 87%, while responses in DLBCL were less than 10%.

We hypothesize that a better characterization of spatial architecture of the tumour microenvironment (TME) in lymphoma will help explain differences in responses to PD1/PDL-1 inhibitors and guide future targeted immunotherapies for these patients. Similar studies in this area have been limited by technical challenges. Single cell techniques such as CyTOF or single cell RNA-seq, rely on tissue disruptions and all spatial information is lost. Current multiplex tissue techniques such as multiplex IHC/IF are limited to 6-8 markers, while spatial transcriptomics lack single cell resolution. In the present study, we characterized TME components, including immunopheno-types, frequency and spatial interaction, in DLBCL using imaging mass cytometry (IMC), which allows high-dimensional, single-cell and spatial analysis of FFPE tissues at sub-cellular resolution. Using a panel of 32 antibodies, IMC was performed 41 tissue microarray cores from 33 DLBCL cases. Using both supervised gating and unsupervised clustering approaches, IMC data were analyzed for relevant immunophenotypes and compared across clinical outcome groups. The TME was primarily composed of CD4+ T-helper cells (13.1% 3 1.9%), CD8+ cytotoxic T cells (10.8% 3 1.1%), CD68+ macrophages (6.3% 3 0.9%), FoxP3+ regulatory T cells (2.7% 3 0.5%), while the bulk of samples were tumor cells 58.1% 3 3.4%. We identify a population of CD8 T cell phenotype co-expressing PD-L1/TIM-3/CCR4 to be enriched in refractory DLBCL. More importantly, we demonstrated a negative association between OS and CCR4/TIM-3-expressing TREG, THELPER and tumour cells, suggesting that DLBCL patients could potentially benefit from TIM-3 and CCR4 inhibitors presently under clinical investigation. To describe the spatial architecture of the tumor microenvironment, we developed a novel clustering approach to group CD8 cells by their distance to tumor and other components of the microenvironment. Using this approach we identified 3 dominant CD8 clusters characterized by their distance to tumor, TREG or macrophages. We were able to demonstrate phenotypic differences in activation (ki-67, granzyme B) or exhaustion (PD-1, TIM3, LAG3) in these different spatial clusters and associate these with response to chemotherapy. Finally, we show that sub-setting our analysis of CD8 phenotypes based on their spatial location to other cells improved our ability to predict overall survival in the cohort. Together, these results show that deep profiling of the immune architecture of the tumor microenvironment is associated with clinical outcomes in DLBCL, and that spatial analysis of immune cells should be explored as a potential biomarker for patients treated with immunotherapies.

Keywords: lymphoma, single cell, microenvironment.

## B157 / Chronic inflammation induced by malnutrition and alcohol consumption regulates fibrosis and the response to immunotherapy in HCC

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Hepatocellular carcinoma (HCC) is one of the most prevalent malignancies worldwide and a leading cause of cancer-related deaths. HCC is initiated by chronic liver inflammation, driven by hepatitis B or C viral (HBV, HCV) infection, alcohol consumption or non-alcoholic fatty liver disease (NAFLD). Despite major gains against HBV and HCV, the epidemic of liver disease continues to grow due to obesity and alcohol abuse. We recently found that high-fat diet (HFD)-induced dysbiosis and inflammation support the development and recruitment of IgA+IL10+PD-L1+ plasmocytes in NASH-afflicted human and mouse livers (1). These plasmocytes cause localized immunosuppression that fosters HCC development by attenuating activation of a protective, tumor-directed, cytotoxic T cell (CTL) response. Liver fibrosis is another outcome of chronic hepatitis, but its exact pro-tumorigenic function remains obscure. The contribution of adaptive immunity to fibrosis and further HCC development has also been elusive. Here we show, that CD8+ T cell ablation enhanced liver fibrosis in MUP-uPA and STAM NASH-induced HCC models, which is consistent with the anti-fibrogenic effect of IFNγ. Similar results were found in the MCD-fed NASH mouse model but not in CCl4-treated animals, whose collagen deposition pattern was different, suggesting that stromal cell types differ in their response to IFNγ (2). Although HFD supports CD8+ T cell infiltration in the liver, alcohol suppresses it. Analysis of the small fraction of HCC-bearing mice that did not respond to anti-PD-L1 treatment revealed immune excluded tumors encapsulated by stromal cells. Moreover, detailed analysis of stromal cells confirmed that they express distinct hepatic stellate cell (HSC) markers. Combination therapy with compounds that inhibit the induction of fibrosis restored the response to immune checkpoint blockade. Our results indicate that the non-responsiveness to anti-PD-L1 treatment correlates with a fibrotic tumor stroma due to CTL exclusion in HCC. Our work provides new insights on how HFD and alcohol regulate adaptive immune cells and thereby affect fibrosis and the response to immunotherapy. Specifically, consumption of alcohol or HFD had a differing effect on the response to anti-PD(L)1 therapy, namely due to their distinct ability to regulate CTL function and induce dysbiosis. Combination therapy with compounds that inhibit the induction of fibrosis can be considered with anti-PD(L)1 therapy in HCC.

Keywords: Immunotherapy, Inflammation, Tumor Microenvironment, Hepatocellular Carcinoma.

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## B159 / Extracellular matrix gene expression and cytotoxic T lymphocyte infiltration in the tumor microenvironment in non-small cell lung cancer

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Immunotherapy has proven to be a powerful anti-tumor therapy, harnessing the body's own immune system to target and kill tumor cells. However, immunotherapy is not successful in all cancer patients due to both intrinsic non-responsiveness and adaptive resistance. Developing predictive biomarkers and understanding mechanisms of resistance are major goals of the immuno-oncology community. The extracellular matrix (ECM), an important factor for promoting tumor growth, survival, and migration of tumor cells, can also act as a physical barrier to prevent immune cell infiltration and promote tumor immune escape. Components of the ECM such as COL11A1, COL4A1, and LOXL2 have been shown to be associated with cancer progression. Furthermore, new data suggests that TGFβ activation leads to up-regulation of ECM genes in cancer-associated fibroblasts and immune suppression. However, it remains poorly understood which cells in the tumor microenvironment (TME) are the sources of ECM gene expression and how they are related to tumor infiltrating cytotoxic T lymphocytes (CTLs). In this study, we employed a highly sensitive and specific RNAscope in situ hybridization (ISH) duplex assay to directly visualize the tissue distribution of cells expressing COL4A1 , COL11A1, LOXL2, and TGFB1 in relation to tumor infiltrating CTLs in non-small cell lung carcinoma (NSCLC). NSCLC tissue microarrays (TMAs) consisting of 63 independent patient FFPE tumor samples were analyzed using this ISH assay with the following probe combinations: Hs-CD8/Hs-IFNG, Hs-CD4/Hs-FOXP3, Hs-LOXL2/Hs-COL4A1, and Hs-TGFB1/Hs-COL11A1. We observed COL4A1 expression in both tumor and tumor-associated stromal cells in different samples. In contrast, COL11A1 was only expressed in tumor-associated stromal cells. Interestingly, high COL4A1 expression was associated with high CD8+ T cell infiltration, whereas high COL11A1 expression was associated with poor CD8+ T cell infiltration. In addition, tumor expression of TGFB1 was positively correlated with COL11A1 expression. These data depict a complex landscape of ECM gene expression and their relationship to T cell infiltration in the tumor and TME. Taken together, these results demonstrate that the RNAscope assay provides a powerful approach to directly examine the interactions between tumor, ECM, and T cell immune infiltration, and offers advantages over immunohistochemistry (IHC) for identifying the cellular sources of secreted proteins such as ECM components in the TME.

Keywords: extracellular matrix, tumor microenvironment, T cell infiltration, RNAscope.

# Tumor antigens

## B160 / Implication of chemo-resistant memory T cells for immune surveillance in patients with sarcoma receiving chemotherapy

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Chemotherapy has improved the prognosis of patients with sarcomas. However, it may suppress anti-tumor immunity. Recently, we reported a novel CD8+ memory T cell population with a chemo-resistance property, "young memory" T (TYM) cells with the high activity of aldehyde dehydrogenase 1 (ALDH). TYM cells were defined by the expression of CD73+CD45RA+CD62L+CCR7+CXCR3+ and CD95- (1). TYM cells memorized virus antigens and some tumor-associated antigens in healthy donors and cancer patients, respectively. In this study, we investigated the proportion and function of TYM cells in peripheral blood of healthy donors and sarcoma patients who received chemotherapy and those who did not. The proportion of TYM cells was significantly decreased in patients compared with that in healthy donors. In healthy donors, anti-EBV CTLs were induced using mixed lymphocyte peptide culture, from not only TYM cells but also TCM and TEM cells. No CTLs directed to tumor-associated antigens were induced. In sarcoma patients who did not receive chemotherapy, in addition to anti-EBV CTLs, CTLs directed to the tumor-associated antigen PBF were induced from TYM, TCM and TEM cells. In sarcoma patients who received chemotherapy, EBV-specific CTLs were induced from TYM cells but were hardly induced from TEM cells. Interestingly, CTLs directed to the anti-tumor-associated antigen PBF were induced from TYM cells but not from the TCM and TEM cells in sarcoma patients who received chemotherapy. The findings suggest that TYM cells are resistant to chemotherapy and can firstly recover from the nadir. TYM cells might be important for immunological memory, especially in sarcoma patients receiving chemotherapy.

Keywords: Memory stem T cell, Chemotherapy, sarcoma.

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## B161 / Novel microfluidic-based immobilization strategy to implement immunopetidomic platforms

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The knowledge of naturally presented HLA class I ligands from the tumor surface (ligandome or immune-peptidome) is essential for designing T-cell mediated cancer therapeutic approaches. Indeed, the generation of specific anti-tumor CD8 T cells relies on recognition of tumor associated antigens (TAA) in the HLA-I complex (1). However, the methodologies for their isolation for the mass spectrometry analysis have been the major bottleneck for a reliable HLA-I peptides characterization (2). To overcame this problem, we set up a new nanotechnology-based immobilization strategy for anti-pan-HLA antibody, to apply for the immuno-affinity purification (IP) of HLA-I complex. The developed technology relies on immobilization of capturing pan HLA-I antibody via the biotin-streptavidin chemistry. Here, biotinylated anti-pan-HLA is immobilized on streptavidin-functionalized surfaces, so that the immune-affinity purification is carried out on customized microfluidic pillars. Our methodology drastically reduces the handling and the timing required for the peptide isolation compared to the reported standard Methods in the field.

In this work, we carefully examined the specificity and robustness of our customized technology for immunopeptidomic workflow. We challenged our novel platform by immunopurifying HLA-I complexes from as low as 107 cells. After the final mild acid elution, HLA-I presented peptides were identified by tandem mass spectrometry and analyzed by in vitro Methods. These results directly imply the opportunity of exploiting microfluidic-based strategy in immunopeptidomic platform and in last analysis in personalized immunopeptidome analysis from individual tumor biopsy to design personalized cancer therapeutic vaccines.

Keywords: Microfluidic, Ligandome, Cancer immunotherapy, tumor antigens.

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## B162 / Correcting neoantigens by accounting for proximal variants using pVACtools

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Recent efforts to design personalized cancer immunotherapies use predicted neoantigens. Typically, to evaluate strong-binding neoantigens from genomic sequencing data, the raw sequencing reads from tumor and normal DNA libraries are aligned to the Human Reference Genome, and somatic variants are identified by comParison of tumor to normal read alignments. The resulting somatic variants of interest are then annotated to predict protein sequence changes and to infer possible neoantigenic peptides. Individual neoantigenic peptides are selected by sliding an amino acid window (usually 8–11-mers) across the variant position to consider each possible register. These peptides are assessed using various algorithms to predict binding affinity to MHC and determine the strongest-binding epitopes. The cancer vaccine design process, from read alignment to variant calling and neoantigen prediction, typically assumes that the genotype of the Human Reference Genome sequence surrounding each somatic variant is representative of the patient's genome sequence, and does not account for the effect of nearby variants (somatic or germline) in the neoantigenic peptide sequence. Because the accuracy of neoantigen identification has important implications for many clinical trials and studies of basic cancer immunology, there is a need for patient-specific inclusion of proximal variants to address this previously oversimplified assumption in the identification of neoantigens.

We evaluated somatic variants from 430 tumors to understand how proximal somatic and germline alterations change the neoantigenic peptide sequence and also affect neoantigen binding predictions. On average, 241 missense somatic variants were analyzed per sample. Of these somatic variants, 5% had one or more in-phase missense proximal variants. Without incorporating proximal variant correction (PVC) for major histocompatibility complex class I neoantigen peptides, the overall false discovery rate (incorrect neoantigens predicted) and the false negative rate (strong-binding neoantigens missed) across peptides of lengths 8–11 were estimated as 0.069 (6.9%) and 0.026 (2.6%), respec-

tively. Thus, for neoantigen identification without PVC in 100 individuals, we can expect that approximately 51 individuals would receive a suboptimal vaccine specifically due to receiving a neoantigen with an incorrect peptide sequence, 23 would receive a suboptimal vaccine specifically due to missing a strong-binding neoantigen, and 62 would receive a suboptimal vaccine due to at least one of these causes.

We also added this improvement as part of a computational toolkit - pVACtools to select and visualize cancer neoantigens. This in silico sequence analysis method aids in each component essential to the vaccine design process. The modular workflow consists of tools for neoantigen prediction from somatic alterations (pVACseq and pVACfuse), prioritization and selection using a graphical web-based interface (pVACviz), and determining the optimal order of neoantigen candidates in a DNA vector-based vaccine (pVACvector).

The results from pVACtools analyses are already being used in cancer immunology studies, including studying the relationship between tumor mutation burden and neoantigen load to predict response in checkpoint blockade therapy trials and the design of cancer vaccines in ongoing clinical trials. We anticipate that pVACtools will make such analyses more robust, reproducible, and facile as these efforts continue.

Keywords: immunogenomics, neoantigens, personalized cancer vaccines, epitope prediction.

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## B163 / Primary human epithelial cells as a model system for the study of inflammation-induced expression of known tumor-associated antigens (TAAs)

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Epidemiological studies have found that acute febrile infections are associated with a greatly reduced life-time risk of many types of cancer (1, 2). Simultaneously, tumor immunologists have shown that immune responses to some of the best-known tumor-associated antigens (TAA) could be found not only in patients with cancer but also in healthy individuals who have never experienced cancer (3, 4). These findings generated a new immunosurveillance hypothesis stipulating that observed cancer-risk reduction relies on the existence of immune memory against self-antigens that were transiently abnormally expressed on infected tissues as disease-associated antigens (DAA) and then later re-expressed on malignantly transformed tissues as tumor-associated antigens (5). We investigated the role of inflammation, that accompanies both infections and carcinogenesis, as the underlying mechanism of generation of DAAs and TAAs. After exposure of normal primary breast epithelial cells to pro-inflammatory cytokines IL-6, IL-1β and TNFα, we used flow cytometry to evaluate expression of well-known TAAs such as carcinoembryonic antigen, HER-2/neu and MUC1. Cells grown in the presence of these three cytokines for 72h showed increased expression of all three antigens plus an increase in the hypoglycosylated, tumor form of MUC1 not usually seen on normal cells. These modifications in the levels of antigen expression were transient and did not last more than two weeks after the removal of the cytokines. We also looked at changes in the global proteome in response to these inflammatory treatments by 2D Difference Gel Electrophoresis (2D-DIGE) combined with mass spectrometry. We identified and confirmed by qRT PCR the over-expression of a dozen of other DAAs that represent potential new TAAs expressed in breast cancer and other tumors. To conclude, inflammatory cytokines known to be present at sites of inflammation are sufficient to induce abnormal expression of self-antigens some of which are known as TAAs. Our findings highlight the need of exploring further the potential of generating immune responses against DAAs, with vaccines that incorporate these antigens, as a strategy for cancer prevention and therapy.

Keywords: inflammation, tumor-associated antigens, MUC1, 2D DIGE.

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## B164 / Identification of breast cancer neoantigens exposed by radiation therapy

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Recent studies have highlighted the key role of neoantigens generated by somatic non-synonymous mutations in tumor response to immunotherapy [1]. In the BALB/c-derived 4T1 mouse model of immune-checkpoint blockade (ICB)-resistant metastatic breast cancer, we have previously shown that tumor-targeted radiation therapy (RT) combined with CTLA4 blockade induces CD8+ T cell-mediated regression of irradiated tumors and inhibits lung metastases [2]. Analysis of the T-cell receptor (TCR) repertoire indicated that unique clonotypes expand in treated tumors, suggesting that tumor rejection involves T cells reactive to a set of tumor-associated antigens that are made available to the immune system by RT [3]. Therefore, we hypothesize that RT increases the expression of genes containing potentially immunogenic mutations and hence promotes priming of neoantigen-specific T cells.

We performed whole-exome sequencing and RNA sequencing of untreated and irradiated (8GyX3) 4T1 cells in vitro to identify tumor-specific neoantigens and determine which ones are upregulated by RT. In addition, these mutations were documented in vivo, in 4T1 tumors harvested before and after treatment (8GyX3 + anti-CTLA4). Dedicated algorithms were used to predict MHC-I and MHC-II-binding epitopes from these mutated genes. Peptides with a predicted affinity <500 nM were synthesized and tested in vitro for binding to H2-Ld or H2-Kd in a MHC stabilization assay using RMA-S cells. Peptides showing stable binding in this assay were used to vaccinate BALB/c mice, followed by challenge with 4T1 cells to test for the induction of protective anti-tumor immunity.

Out of 309 total mutations initially identified in 4T1 cancer cells, 22 predicted MHC-I-binding epitopes were tested in vitro and 6 of them were confirmed to bind to H2Ld or H2Kd. For MHC-II, we identified two I-Ad-predicted binders, which were tested in vaccination experiments. Two MHC-I and one MHC-II neoepitopes were immunogenic, as assessed by IFNγ/TNFα response after T cell re-stimulation. These three neoepitopes were encoded by genes upregulated by RT. Although a vaccine based on these three neoepitopes was not sufficient to inhibit tumor growth, a significant

growth delay was seen when vaccination was combined with tumor-targeted RT. TCR repertoire analysis of these tumors revealed significant vaccination-related differences in the frequency of T cell clones, independent of clones specific for the dominant AH1 epitope (derived from the gp70 shared tumor antigen). In addition, in vivo killing experiments demonstrated a potent cytolytic activity of T cells from vaccinated mice towards one of the MHC-I restricted neoepitopes. Further analyses are ongoing to understand the contribution of these neoantigens to T cell-mediated tumor rejection in the context of targeted RT.

In conclusion, these data provide initial proof-of-principle evidence that RT can expose existing neoantigens to the immune system.

Keywords : neoantigens, radiation, breast cancer.

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## B165 / Splicing patterns in SF3B1 mutated uveal melanoma generate public immunogenic epitopes

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To directly or indirectly induce an immune response towards tumor-specific neo-epitopes is a promising therapeutic strategy in oncology. Differentiation or oncotestis antigens are shared between patients and have been targeted in many clinical trials with mixed successes. Indeed, the high avidity T cell repertoire that recognize these antigens may not be available after central and peripheral tolerance. In addition, generating an efficient immune response toward this class of antigens may lead to auto-immunity. In contrast, passenger mutations produce tumor-specific neo-epitopes, which are, however, specific for each patient making vaccine strategies targeting them logistically very cumbersome. To identify neo-epitopes that would be both specific of the tumor and shared between patients is therefore an important goal.

Mutations in splicing factors change the splicing pattern of many transcripts, and by consequences of many protein products, generating neo-epitopes through the inclusion of intron sequences associated or not with frameshift in the following exon. The resulting neo-peptides are germinally encoded leading to neo-epitopes shared between patients with common HLA haplotypes. Although proposed for several years, this has not been demonstrated.

Here, we show that mutations in the splicing factor, SF3B1, which is mutated in 20% of uveal melanoma (UM), generate public neo-epitopes that induce a memory response in the metastatic UM patients whose tumor harbors this mutation. SF3B1 mutations modify the mRNA splicing pattern in about 1060 junctions in UM tumors. From these junctions, in silico analysis predicted 46 neo-epitopes with high avidity for HLA-A2. The 46 corresponding tetramers identified variable frequency of specific CD8 T cells in 4 healthy donors and 4 patients with SF3B1 wild-type UM. These T cells displayed a naive (CD45RA+CCR7+) phenotype. In contrast, in 8 patients with mutated SF3B1 UM, the frequency of specific T cells for 8 epitopes was increased (up to 0.4 % of CD8 T cells) as compared to the controls and these cells were memory (CD45RA-CCR7+/-). For one neo-epitope studied in the blood of one patient, single cell repertoire analysis of the tetramer-positive T cells identified recurrent TCRs that were also found in the tumor. T cells clones specific for several SF3B1mut related epitopes specifically recognized SF3B1-mutated but not wild type tumors. Thus, SF3B1 mutations lead to many public neo-epitopes expressed by UM tumors in a way recognizable by CD8 T cells. This proof of concept opens the possibility of tumor-specific therapeutic vaccines targeting these public epitopes or of bi-specific reagents recognizing both the specific HLA-A2:peptide complexes and T cells. Since SF3B1 mutations are observed in other tumor types, these therapeutic strategies could be generalized to other cancers harboring low number of passenger mutations but mutated splicing factors.

Keywords : Splicing factors, Uveal melanoma.

## B166 / The antigenic landscape of glioblastoma : Refining the targets for immunotherapy

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Glioblastoma is the most common and most malignant primary brain tumor with a poor prognosis despite surgery and chemo-radiotherapy. A major challenge is the fact that tumor cells infiltrate the healthy brain tissue, precluding complete surgical resection. Thus, innovative treatment approaches for glioblastoma are urgently needed. Among these, immunotherapy receives increasing attention, also because glioblastoma is paradigmatic for cancer-associated immunosuppression. We provide a comprehensive analysis of the antigenic landscape of glioblastoma using a multi-omics approach including ligandome mapping of the human leukocyte antigen (HLA) ligandome, next generation sequencing and characterization of tumor-infiltrating lymphocytes (TIL) using mass cytometry and ultra-deep sequencing of the T-cell receptor (TCR). HLA class I and class II ligands of 24 isocitrate dehydrogenase 1 wildtype glioblastoma samples and 10 autologous primary glioblastoma cell lines were mapped using immunoaffinity purification followed by sequencing using liquid chromatography coupled with tandem mass spectrometry. To identify tumor-exclusive ligands, an HLA ligandome database containing normal tissues (n > 418) was used as reference. We found 11, 496 glioblastoma exclusive HLA class I ligands. Interestingly, 2, 064 ligands were shared with cell lines and 3, 754 were presented on at least 2 glioblastoma samples. On the source protein level 239 glioblastoma exclusive proteins were identified ; among them 54 were also present in cell lines and 178 were presented on at least 2 glioblastoma samples. For HLA class II ligands the analysis revealed 11, 870 glioblastoma exclusive peptides with 444 being in common with cell lines. Interestingly, 3, 420 glioblastoma-exclusive peptides were presented on at least 2 glioblastoma samples. On the source protein level 278 glioblastoma exclusive proteins were found ; among which 18 were present also in cell lines and 82 were presented on at least 2 glioblastoma samples. Moreover, whole-exome sequencing and RNA sequencing from 13 tumor samples was performed with the aim to predict neoantigens. On average 5, 662 somatic missense effects were identified per patient (min : 4, 258 ; max : 7, 479). Candidate peptides are grouped into (i) in silico predicted neoepitopes, (ii) tumor-exclusivity on HLA, (iii) gene expression (e.g. cancer testis antigens). Top-ranking candidates from each group will be tested with regards to their immunogenicity in an autologous setting (TIL, peripheral blood mononuclear cells, patient derived tumor cells). Finally, the peptide and immunogenicity data is correlated with the immune phenotype of the TIL compartment as well as the TCR repertoire of the sample.

Keywords: antigenic landscape, glioblastoma.

## B167 / Integration of In Vivo and In Silico Models for Predicting Neoantigens Driving Responses in Melanoma Immunotherapy

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Immune checkpoint blockade (ICB) can induce response in malignant melanoma. However, recurrent disease still occurs in the majority of cases. It is urgent to identify the determinants of responses to ICB and explore how they may serve as therapeutic targets. We hypothesize that immunogenic neoantigens drive melanoma response to ICB. To address this, we incorporated preclinical and computational modeling to identify response-driving neoantigens. We have developed four syngeneic mouse models that represent the pathological and immunological diversity of BRAF-mutated, RAS-mutated, and triple wildtype human melanomas. In preclinical studies, two of the models are resistant to anti-CTLA-4 while the other two are sensitive. By comparative analysis of the exome and RNA sequencing results of the four models, we found that the sensitivity of tumors to anti-CTLA-4 is associated with the following features of neoantigens: (1) number; (2) expression level; and (3) MHC binding measured by Mouse Harmonic-mean Best Rank (MHBR) [1, 2]. We generated a model to give each neoantigen an immunogenic score (IGS) based on these three parameters, and the aggregated IGS of all the neoantigens is used to predict response of individual melanoma to immune checkpoint inhibitors. We validated the aggregated IGS in data from melanoma patients receiving ipilimumab and nivolumab and revised the model accordingly. For functional validation, we generated a library of neo-epitopes from the predicted immunogenic neoantigens. An ICB-resistant mouse melanoma expressing the library was subjected to anti-CTLA-4 treatment, and the neo-epitopes responding to anti-CTLA-4 were identified by sequencing. Interestingly, we found the validated high-IGS neoantigens were derived from mutations involved in important functions, including cell cycle checkpoint and apoptosis. Our results imply that high-IGS neoantigens derived from functional driver mutations may induce immune response, driving response to ICBs. *MD and CPD contribute equally to this study. **Correspondence addressed to HC and GM.

Keywords: Melanoma, Immune checkpoint blockade, Neoantigens, Machine learning.

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## B168 / Ribosome profiling predicts novel unannotated open reading frames that contribute peptides to the MHC class I immunopeptidome in healthy and cancer cells and can be a source of neoantigens

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Cancer-specific neoantigens, derived from somatic mutations, presented on the MHC class I, and recognized by the immune system, have emerged as an important target to drive immunotherapy. Currently, neoantigen predictions are based on mutations detected by whole exome sequencing, which covers a pre-determined set of annotated protein-coding genomic regions, and often falls short for patients with few somatic mutations.

Ribosome profiling (Ribo-seq) has suggested a plethora of translated novel unannotated open reading frames (nuORFs). They can be derived from alternative out-of-frame ORFs overlapping annotated protein-coding ORFs, untranslated regions (UTRs) of annotated protein-coding transcripts (5' uORFs, 3' dORFs), long non-coding RNAs (lncRNAs) and pseudogenes. We hypothesized that nuORFs can provide another source of neoantigens in cancer. In particular, we focused on nuORFs in two categories: 1) expressed in healthy and cancer cells, and have acquired cancer-specific somatic mutations; 2) upregulated in or specific to cancer cells.

To explore this hypothesis, we performed Ribo-seq on primary healthy and cancer cells and cell lines from melanoma, glioblastoma, colon carcinoma and chronic lymphocytic leukemia. Using this expansive dataset, we performed hierarchical ORF prediction analysis to build a database of highest confidence predicted translated nuORFs across healthy and cancer cell types.

To determine if peptides from nuORFs can be a source of antigens, we performed MHC class I immunoprecipitation and liquid chromatography with tandem mass spectrometry (LC-MS/MS) on the same samples. We also searched our collection of mono-allelic MHC class I immunopeptidome MS spectra from 92 common HLA alleles against our pan-sample nuORF database. Using a combination of these approaches, we found HLA-presented peptides derived from thousands of nuORFs: out-of-frame, within 5' and 3' UTRs, lncRNAs, pseudogenes, and other RNA species. The HLA-binding motifs of peptides from nuORFs correspond to the expected motifs for given HLA types.

Previous analyses of the whole proteome have been able to identify only a handful of unannotated short ORFs. Thus, we analyzed whole proteomes from the same cells as used for Ribo-seq and MHC class I immunopeptidome analyses to compare them directly. Strikingly, peptides from short nuORFs are dramatically over-represented in the MHC class I immunopeptidome as compared to the whole proteome.

To identify tumor-specific mutations in nuORFs, we performed whole genome sequencing on patient-matched healthy and cancer cells and mapped somatic mutations to nuORFs. We identified somatic mutations supported by Ribo-seq and RNA-seq reads in translated nuORFs, suggesting that they can be additional sources of neoantigens in cancer. Finally, to identify nuORFs upregulated in or specific to cancer cells, we compared translation levels of nuORFs between healthy and cancer cells of the same origin. We found sets of nuORFs of various categories highly upregulated in, or specific to cancer cells, suggesting that they can also give rise to neoantigens.

In conclusion, we identify hundreds of nuORFs of different types that are unequivocally translated and contribute peptides to the MHC class I immunopeptidome in healthy and cancer cells. We show that somatic mutations in nuORFs have the potential to expand the neoantigen repertoire. Finally, we identify nuORFs that are differentially translated or uniquely expressed in specific cancer types, suggesting that they can further contribute to the neoantigen pool. Thus, an expanded search strategy for neoantigens that combines Ribo-seq and proteomic analyses significantly broadens potential targets for therapy, particularly for cancers with low somatic mutation load.

Keywords : ribosome profiling, neoantigen, Ribo-seq.

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## B169 / Neoantigen prediction for cancer vaccine across entities

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Different strategies of cancer immunotherapy have shown outstanding success in a number of patients across various tumor types. Cancer genome-based identification of patient-specific neoantigens plays an important role in cancer immunotherapy, e.g. vaccine strategies. The expression of neoantigens by tumor cells allows the immune system to distinguish cancer cells from normal cells and, potentially leads to activation of the patient's own immune effector cells to specifically eradicate tumor cells. However, only a small fraction of genomic mutations may lead to immunogenic neoantigens and thus a reliable prediction of immunogenic neoantigens is required. We developed a pipeline to determine the patient´s HLA type and to predict MHC class I and II neoantigens based on the analysis of the next-generation sequencing data. Our pipeline is able to predict neoantigens from nonsynonymous mutations, small insertions/deletions and gene fusions, and has already been successfully applied to predict neoepitopes in various cancer patients. Among 24 vaccinated neopeptides, more than 80% can either expand pre-existing T-cell responses or induce new T-cell specificities after vaccination in cancer patients (n=4) across tumor entities. Importantly, we demonstrate that neopeptides arising from fusion genes can also induce T-cell responses. Currently, this pipeline is being optimized and further experimental validation is conducted to identify which fusion-derived neoepitopes among the vaccinated neopeptides can stimulate T cell responses. Perceptively, we aim to efficiently translate the neoantigen-based strategy to clinical application, e.g. TCR-specific engineered T-cell therapy.

Keywords : Neoantigen, Fusion, Immunotherapy, Cancer vaccine.

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Ko²alo²lu, Z., Zörnig, I., Halama, N., Kaiser, I., Buchhalter, I., Grabe, N.... & Jäger, D. (2016). Identification of immunotherapeutic targets by genomic profiling of rectal NET metastases. Oncoimmunology, 5(11), e1213931.

## B170 / HLA ligandome analysis reveals an antigen processing signature required for HLA class I presentation and CD8+ T cell responses

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CD8+ T cells recognize peptides displayed by HLA class I molecules, discriminating cancer cells. Difficulty in predicting HLA class I ligands is attributed to the complexity of the antigen processing pathway across the cytosol and the endoplasmic reticulum (ER). Here, we captured natural HLA class I ligands displayed by multiple types of cancer cell lines using HLA ligandome analysis that employs mass spectrometry, and analyzed the imprints of antigen processing. The comprehensive analysis of source-protein sequences flanking the ligands revealed that the frequency of proline at amino acid positions 1-3 upstream of the ligands was selectively decreased. The depleted proline signature was the strongest among all the upstream and downstream profiles. Experiments using live cells demonstrated that the presence of proline at upstream positions 1-3 attenuated CD8+ T cell responses against a model cancer antigen. Other experiments in which N-terminally flanking antigen precursors were confined in the ER demonstrated an inability to remove upstream prolines regardless of their positions, suggesting a need for synergistic action across cellular compartments for making the proline signature. Our results highlight an antigen processing signature that could affect HLA class I peptide repertoire formation and CD8+ T cell responses.

Keywords : Tumor antigens.

References :
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## B171 / Immunogenicity of HLA class II-restricted neoantigens derived from driver mutations

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Recently, increasing attention has been paid to neoantigens that are derived from somatic genetic mutations specifically present in cancer cells. Since they can be recognized as non-self by the immune system, they are expected to induce stronger immune responses than non-mutated self-antigens. In particular, since "driver mutations" that are directly involved in malignant processes are frequently shared by patients with various types of cancers and do not disappear easily by immune escape, they could represent appropriate off-the-shelf targets for cancer immunotherapy.

In the current study, we evaluated the immunogenicity of 10 well-known driver mutations, including KRAS-G12D, KRAS-G12V, KRAS-G12C, KRAS-G12R, KRAS-G13D, NRAS-Q61K, NRAS-Q61R, PIK3CA-E545K, PIK3CA-H1047R, and C-Kit-D816V, which are frequently expressed in various cancers using peripheral blood mononuclear cells from healthy donors (n = 25). Of the 10 synthetic peptides (27 mer) derived from these mutations, the six peptides from KRAS-G12D, KRAS-G12R, KRAS-G13D, NRAS-Q61R, PIK3CA-H1047R, and C-Kit-D816V induced T cell responses. In particular, more than 10% of the donors showed immune responses to PIK3CA-H1047R, C-Kit-D816V, KRAS-G13D, and NRAS-Q61R. All six peptides induced HLA class II-restricted CD4+ T cell responses ; notably, PIK3CA-H1047R contained at least two different CD4+ T cell epitopes restricted to different HLA class II alleles. In addition, PIK3CA-H1047R and C-Kit-D816V induced antigen-specific CD8+ T cells as well, indicating that they might contain both HLA class I- and class II-restricted epitopes.

Our findings suggested that frequent driver mutations are not always less immunogenic, since six of 10 well-known driver mutations induced specific T cell responses. Since the identified neoantigens might be shared by patients with various types of cancers, they have the potential to be promising off-the-shelf cancer immunotherapy targets in patients with the corresponding mutations.

Keywords : neoantigen, driver mutation, HLA class II.

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Iizumi S, Ohtake J, Murakami N, Kouro T, Kawahara M, Isoda F, Hamana H, Kishi H, Nakamura N, Sasada T. Identification of Novel HLA Class II-Restricted Neoantigens Derived from Driver Mutations. Cancers (Basel). 2019 Feb 24 ;11(2). pii : E266. doi : 10.3390/cancers11020266.

## B172 / T cell recognition of novel shared breast cancer antigens is frequently observed in peripheral blood of breast cancer patients

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Purpose: Advances within cancer immunotherapy have fueled a paradigm shift in cancer treatment, resulting in increased cancer types benefitting from novel treatment options. Despite originally being considered an immunologically silent malignancy, recent studies encourage the research of breast cancer immunogenicity to evaluate immunotherapy as a treatment strategy. However, limiting options for antigen-specific, targeted strategies. Aromatase, never in mitosis A-related kinase 3 (NEK3), protein inhibitor of activated STAT3 (PIAS3), and prolactin are known as upregulated proteins in breast cancer. In the present study, these four proteins are identified as novel T cell targets in breast cancer.

Experimental Design: From the four proteins, 147 peptides were determined to bind HLA-A*0201 and -B*0702 using a combined in silico/in vitro affinity screening. T cell recognition of all 147 peptide-HLA-A*0201/-B*0702 combinations was assessed through the use of a novel high-throughput method utilizing DNA barcode labeled multimers.

Results: T cell recognition of sequences within all four proteins was demonstrated in peripheral blood of patients, and significantly more T cell responses were detected in patients compared to healthy donors for both HLA-A*0201 and -B*0702. Notably, several of the identified responses were directed towards peptides, with a predicted low or intermediate binding affinity. This demonstrates the importance of including low-affinity binders in the search for epitopes within shared tumor associated antigens (TAAs), as these might be less subject to immune tolerance mechanisms.

Conclusion: The study presents four novel TAAs containing multiple possible targets for immunotherapy of breast cancer.

Keywords: Breast cancer, Immune monitoring, Tumor associated antigens, T cell recognition of shared antigens.

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## B173 / Inter-myeloid cell synaptic tumor antigen dispersal impacts anti-tumor T cell priming

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Immune responses to cancer require the movement of tumor-derived antigens into the tumor draining lymph node (tdLN) where T cells can then be primed by a diverse set of dendritic cell (DC) populations. Previous work has shown that migratory DC populations are required to carry tumor antigens to the tdLN and more broadly to disseminate tumor antigens to tdLN resident DC populations, but the biological mechanism for this has been unclear (1). Peptides injected in the context of vaccination can directly drain to the LN for sampling by resident myeloid cells (2, 3) whereas migratory cells are required to carry antigen to the LN in other physiological challenges such as infection or tolerance, and the antigens are then subsequently acquired by resident DC (4–10). In the context of an actively growing tumor, much less is known, competing hypotheses from other systems suggest migratory DC may: 1. Undergo apoptosis and are taken up by resident cells (7); 2. Secrete exosomes containing antigen for subsequent stochastic re-uptake (11); 3. Secrete soluble antigens into the LN interstitium (12) or 4. Transfer entire peptide-MHC complexes, in a process termed cross-dressing (13). By tracking tumor antigen dispersal to and within the tdLN we visualize the contact-dependent mechanism by which antigen is propagated, namely: migratory DC bring antigen to the tdLN contained within a distinct collection of vesicles, and these are discretely transferred to other myeloid cells through a synaptic interface. In vitro transfer assays and in vitro and in vivo imaging all demonstrate that this handoff process occurs via direct vesicular transfer from one myeloid cell to another at transient synaptic contacts formed between myeloid cells. Pulse-chase experiments reveal a sequential dissemination of antigen to the cells of the tdLN and that cells to which these vesicles were transferred account for the majority of LN priming. By honing in on the DC that actually bear antigen, we demonstrate that this transfer ultimately allows diversification of T cell differentiation. These findings are fundamental to understanding how T cell recognition of presented tumor antigens ('signal one') is regulated and can be targeted. It further provides an understanding of when and where existing 'signal two' checkpoint drugs may be engaged for cancer immunotherapies.

Keywords: dendritic cells, lymph nodes, live-imaging.

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## B174 / A determinant leading to CD8+ T-cell induction in neoantigens with a single amino acid substitution

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Factors that define immunogenicity of neoantigens consist of multiple factors and yet remain elusive. We previously analyzed the peptide landscape of a colon cancer line (HCT15/b2m) by means of HLA-A24 ligandome analysis, and detected a 9-mer neoantigen (AKF9) as well as its wild-type counterpart (ANF9). The AKF9 was encoded by the ubiquitously expressed AP2S1 gene carrying a missense mutation that gives rise to a single amino-acid substitution (Asn>Lys) at P8. In contrast to the wild-type ANF9, the neoantigen AKF9 proved its capacity to induce specific T-cell responses across healthy donor PBMCs, which induced CD8+ T cells exhibiting specific and considerably high cytotoxicity. Because ANF9 was repeatedly detected across healthy donor cells, we hypothesized that the non-selfness inducing the T-cell responses of AKF9 is attributed to an amount of the structural change between Asn and Lys. In order to investigate the hypothesis, we prepared AKF9 variants (AIF9、ADF9、AHF9、ASF9、ATF9、AYF9) that can be generated by random missense mutations. After peptide stimulation to 5 healthy donor PBMCs, we found that the effector T-cell induction rates determined by IFN-gamma production were not homogeneous: AKF9 and AHF9 scored higher induction rates. Interestingly, the variants with higher induction rates also showed higher epitope volume change (EVC) scores, which were the volume differences between the variants and wild-type peptides predicted using 3D modeling of peptide-HLA-A24 complexes. Prediction of pHLA structures is yet challenging; nevertheless, our finding may suggest its role upon T cell induction efficiency and contribute to prediction of quality neoantigens.

Keywords: Tumor antigens.

## B175 / Identification of a new type of neoantigen derived from splice peptides

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When a gene mutation occurs in a cancer cell, the antigen derived from the mutant protein encoded by that gene is presented. The antigen generated by this genetic mutation is called a neoantigen. It is not found in normal cells and is unique to cancer cells. Therefore, it serves as a target of the immune system, which eliminates non-self cells, and its expression plays an imperative role in determining the success or failure of cancer immunotherapies, including immune checkpoint inhibitor therapy. The proteasome can cut and paste peptide sequences, thereby releasing peptide antigens that do not correspond to the original protein sequence. This proteasome-catalyzed peptide splicing (PCPS) has long been considered to occur only rarely. It has recently been reported that the PCPS is presented on the surface of many cells (Liepe J, et al. Science. 2016.).

Although little specific example of a proteasome-generated spliced epitope carrying a mutated amino acid has reported till date, we considered that a mutation-derived spliced epitope could be utilized as a therapeutic target as a neoantigen and probed into it. We independently created a database for genetic mutation-derived spliced epitope detection. As a result of exhaustive exploration of antigenic epitopes presented on cancer cells by proteogenomic HLA ligandome analysis, we succeeded in identifying a candidate spliced epitope derived from gene mutations. Furthermore, we confirmed the induction of cytotoxic T cells response to the mutation-derived spliced peptide in peripheral T cells, and demonstrated the immunogenicity of itdem. This genetic mutation-derived spliced epitope is a novel type of neoantigen and is anticipated to be a novel target antigen for cancer immunotherapy.

Keywords: Tumor antigens.

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Liepe J, Marino F, Sidney J, Jeko A, Bunting DE, Sette A, et al. A large fraction of HLA class I ligands are proteasome-generated spliced peptides. Science 2016;354:354–8.

## B176 / Comparing the predictive power of proposed neoantigen descriptors on a standardized analysis platform

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Neoepitopes are mutated peptide sequences that are specific to a patient's tumor. Neoantigen-specific T cells recognize these sequences while eliciting an anti-tumoral response. With the success of checkpoint inhibition-based therapies, neoantigens became a focus of attention, as their existence has been shown to be predictive for therapy benefit [1]. Besides other factors, the response to checkpoint inhibitor treatment is crucially dependent on recognition of neoepitopes. Furthermore, their tumor specificity makes neoantigens a promising target for individualized cancer immunotherapy and we could already demonstrate that patient-specific neoepitope vaccination can even induce antigenic de novo responses [2]. Hence, the detection and prediction of neoantigens is of great importance for the field of cancer immunotherapy.

Next-Generation Sequencing (NGS)-based exome sequencing of tumor and matched normal tissue is the state-of-the-art strategy to detect mutation profiles in tumor samples. However, it is still challenging to predict which mutation might give rise to an immunogenic neoepitope. Although several potential neoantigen features for immunogenicity prediction and target prioritization have been published within the last years, these features have been established employing diverse strategies, with respect to data types, data sets and data analysis tools (e.g. [3], [4]). A standardized and comparative performance evaluation of these proposed descriptors is still missing.

Therefore, we collected public sequencing and outcome data from checkpoint inhibitor studies and processed these data in a best practice way with our in-house developed iCaM (individualized cancer mutation) pipeline to detect patient-specific mutated peptide sequences (e.g. [1], [5]). We annotated the identified candidates with proposed neoantigen features and benchmarked their predictive power. Here, we present a comparative evaluation of published neoepitope properties by correlating each neoantigen feature with patient outcome after immunotherapy as a measure for anti-tumoral immune response. With our standardized processing and analysis approach, we observe a particularly heterogeneous nature of neoantigen descriptors across investigated patient cohorts.

Keywords: neoantigen, neoepitope prediction, benchmarking.

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## B177 / Integrated proteogenomic deep sequencing and analytics accurately identify the tumor non-canonical immunopeptidome

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Efforts to precisely identify human leukocyte antigen presented peptides (HLAp) capable of mediating T cell based tumor rejection still face important challenges. Recent reports suggest that cancer non-canonical (nonc) HLAp derived from aberrant translation and the presentation of presumably non-protein-coding regions could serve as immunogenic targets. However, their identification is limited by the reliance on highly sensitive and accurate mass-spectrometry (MS)-based proteogenomics approaches.

Here, we present a MS-based analytical pipeline that characterizes the noncHLAp repertoire, which was validated on seven patient-derived melanoma cell lines and two lung cancer samples

with matched normal tissues. The workflow incorporates whole exome sequencing, both bulk and single cell transcriptomics, ribosome profiling, and a combination of two MS/MS search tools with group-specific false discovery rate calculations for accurate HLAp identification.

Overall, this led to an in-depth repertoire of 60, 320 unique protein-coding HLAIp and 11, 256 HLAIIp. Importantly, for the nonc space, the combination of two MS/MS search tools enabled the confident identification of more than 400 shared and tumor-specific noncHLAp derived from the expressed lncRNAs, transposable elements and alternative open reading frames (ORFs). These noncHLAIp were highly enriched with HLA-binders (median: 90%). Furthermore, the presented dataset provides a comprehensive insight into the relationship between expression, translation and HLA presentation from canonical and nonc sources. We observed that the immunopeptidome is better captured by the translatome than the transcriptome (two sided p-value < 10e-16), and that de novo Ribo-Seq assembled protein sequence databases led to the discovery of additional noncHLAIp. While source nonc genes were often expressed at low levels, 42 (out of 85 tested) noncHLAIp were confirmed by targeted MS, by which synthetic heavy isotope-labelled peptides were spiked into the peptidomic sample. We further validated through ribosome profiling the translation of the correct ORF in around 20% of the nonc genes that gave rise to the noncHLAIp. Moreover, a handful of noncHLAIp were experimentally confirmed through targeted MS to be shared across tumors, highlighting their potential relevance in the rapid treatment of a large number of patients when compared to private neoantigens. Lastly, we show CD8+ T cell specific recognition of a novel noncHLAIp derived from a downstream ORF of the ABCB5 gene, demonstrating its potential immunogenicity. In sum, this comprehensive analytical platform holds great promise for the discovery of novel cancer antigens for cancer immunotherapy.

Keywords: Immunopeptidomics, non-canonical HLA peptides, Proteogenomics, Mass Spectrometry.

## B178 / Immunopeptidomics of colorectal cancer organoids reveals a sparse HLA class I neoantigen landscape and no improvement with interferon or MEK-inhibitor treatment

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Microsatellite stable (MSS) colorectal cancers (CRCs) are largely refractory to immune-checkpoint inhibiting therapies (1, 2). We aimed to assess potential reasons for the poor immunotherapy sensitivity by applying mass spectrometry (MS) immunopeptidomics to investigate the number of neoantigens presented on MSS CRC cells. Patient Derived Organoids (PDOs) are thought to better represent CRCs in patients than traditional cell line models; therefore, our approach used PDOs to map the CRC immunopeptidome. MSS PDOs from four chemotherapy refractory CRCs and one treatment-naïve CRC were expanded with a novel culture technique utilising low-concentration (2%) matrigel (3). This allowed for expansion to 100 million cells per replicate, facilitating high-sensitivity MS analysis of HLA class I and II peptide ligands (4). Exome sequencing was used to ascertain the mutational profile of each PDO. MS immunopeptidomics and RNA sequencing were performed on untreated, interferon gamma (IFNγ)- and MEK-inhibitor (trametinib) treated PDOs to correlate gene expression changes with immunopeptidome changes. An average of 9, 936 unique HLA class I-bound ligands per PDO were identified by MS, comparing favourably against similar studies and hence confirming high sensitivity of the technique. Low peptide diversity in one PDO was likely explained by loss of heterozygosity of the HLA locus. Importantly, only 3 of the 612 non-silent mutations across the 5 PDOs generated an HLA class I-binding neoantigen that was detectable by MS. No neoantigens were detected on HLA class II. IFNγ treatment did not increase HLA class I peptide presentation significantly, with an average of 1.07-fold increase, however it did qualitatively change the immunopeptidome. IFNγ treatment did increase HLA class II peptide presentation, at an average of 10.05-fold. However, no additional neoantigens became detectable upon IFNγ treatment despite the increases in HLA class I and II, immunoproteasome subunits and antigen processing machinery detected by RNA sequencing. Trametinib had no consistent effect on HLA expression, or the immunopeptidome. Computational neoantigen prediction with NetMHCpan4.0 predicted 304 of the 612 mutations across the five PDOs to encode for neoantigens that strongly bind HLA class I (5). 196 of these neoantigens were derived from genes that were expressed in our PDOs based on RNA-sequencing. The discrepancy between 196 predicted neoantigens in expressed genes vs. 3 neoantigens that were actually detected by MS immunopeptidomics suggests a major overestimation by computational prediction. Presentation of cancer/testis antigens (CT) on HLA molecules was detected in only two of five PDOs, each presenting one peptide derived from one CT antigen. Neither IFNγ nor trametinib enhanced the presentation of CT peptides. We showed that CRC PDOs can be expanded to large cell numbers for high sensitivity neoantigen landscape interrogation by MS. Although MS immunopeptidomics has sensitivity limits and biases, making it likely to have underestimated the true neoantigen load, the detection of so few neoantigens suggests a sparse neoantigen landscape in MSS CRC. This may explain the lack of immune checkpoint-inhibitor activity in most MSS CRCs. MEK-inhibitors recently failed to improve checkpoint-inhibitor efficacy in CRC and the observed lack of HLA upregulation and of improved peptide presentation may explain this. The ability to perturb PDO cells with drugs or genetic manipulation should lead to novel approaches for improving neoantigen presentation. PDO immunopeptidomics will furthermore be useful to train and improve computational neoantigen prediction algorithms which is important for personalized immunotherapy approaches and particular for neoantigen vaccination technologies.

Keywords: Mass Spectrometry (MS), Neoantigens, Immunopeptidomics, Patient-derived organoids (PDOs).

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## B179 / Improving indirect maleimide based ELISA for the detection of antibodies against tumour-specific antigens

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Indirect ELISAs are a commonly used assay, which involve a two-step process for detection, designed to detect the presence of a protein, an antigen or antibody of interest. It does, however, come with limitations and results are often misinterpreted due to lack of appropriate controls and a poor understanding of what is being detected [1]. In particular, the detection of specific circulating antibodies present in serum/plasma to defined antigen bound to a plate has been an assay fraught with methodological misconstruction [1, 2, 3]. With a focus on the common issue of non-specific binding by the antibodies being detected to the plastic ELISA plate, we set out to optimise the indirect ELISA technique

using maleimide-activated plates. These plates bind target peptide antigens by a cysteine residue. We have shown that human plasma contains IgG capable of binding to a non-coated plate to produce a high signal, which is sometimes higher than that of a coated well. Here, we demonstrate that in order to ensure an indirect ELISA using maleimide plates on human plasma/serum are as precise as possible, it is important to: a) include positive controls, negative controls and plate loading controls; b) perform titration experiments to define the concentrations of each component (e.g. the linear peptide antigen, the dilution of plasma used or the concentration of primary and secondary antibodies, dependent on the design of the indirect ELISA); and c) carefully select the choice of blocking agents. Together, these findings establish these steps as vital to improve this type of ELISA so that researchers can ensure the specificity of their signal and prevent misinterpretation of results. The intention of optimisation of this assay is for the detection of tumour-specific humoral immunity. This indirect ELISA method will be a good tool to identify novel tumour-specific candidate targets for immunotherapy and evaluate patient diagnosis or prognosis in clinical applications.

Keywords: ELISA, B cells, IgG, tumour antigens.

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## B180 / Benchmarking the foreign antigen space of human malignancies

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Mutational load varies widely between malignancies and has been used as a proxy for the immunological foreignness of human cancers. However, without well-defined reference points it is difficult to determine which human tumors can be considered sufficiently foreign to the T-cell-based immune system. We established a neo-antigen prediction pipeline that processes single nucleotide variants, indels and gene fusion events and established its precision in identifying T-cell-recognized antigens. We used this pipeline to benchmark the immunological foreignness of human cancers against pathogens for which T-cell control has been well documented. We demonstrate that up to 50% of tumors, spanning 25 sites of origin, are more foreign than these pathogen benchmarks. We also demonstrate that the neo-antigen repertoire of treatment-naïve tumors is not detectably influenced by immune editing. Finally, we report varying potential in neo-antigen generating capacity between mutational processes. These data suggest that immunotherapeutic strategies that enhance activity of the endogenous T-cell compartment may be of value for a large fraction of human cancers.

Keywords: neo-antigens, neo-epitope prediction, immune editing.

## B181 / Targeting prostate-specific membrane antigen: from phage display to nanomolar conformation-selective antibody mimetic

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Discovery of novel ligands of pharmacologically relevant targets is an essential part of drug development. A number of selection Methods can be utilized in the search for binders of pertinent macromolecules such are e.g. cancer antigens. In this article, we describe a development of fully synthetic antibody mimetic selectively targeting specific conformation of Prostate-specific Membrane Antigen (PSMA), which this transmembrane metalloprotease adopts in its free uninhibited state [1]. PSMA, also known as Glutamate carboxypeptidase II, is overexpressed in prostatic malignancies and serves as a validated diagnostic and a potential therapeutic marker.

The design of our mimetic is based on a discovery of new bicyclic peptide binder selected via phage display. An fd phage library displaying bicyclic peptides with variable loop lengths, established and kindly provided by Dr. Heinis (EPFL Lausanne) [2], was screened for a phage-binder of the uninhibited conformation of the extracellular domain of PSMA. The lead hit was identified using a repeated three-round selection followed by a next generation sequencing (lead hit composed approximately 75% of the pool). The candidate peptide was synthesized and an antibody mimetic, so-called iBody [3], was created by conjugation of the peptide to a biocompatible, water soluble polymer carrier. The iBody was additionally accessorized with fluorophore and affinity anchor molecules for easy detection and immobilization respectively. The peptides function as targeting ligands for delivery of the whole polymer conjugate to its intended destination.

Within the scope of our work, we assess and compare the binding properties of the bicyclic peptide binders and the peptide-functionalized iBodies. We test the iBodies as antibody substitutes in various Methods (e.g. confocal microscopy, flow cytometry, ELISA, SPR) and demonstrate that both, the affinity and the specificity of the binding, are significantly improved by the conjugation of the peptides to the hydrophilic carriers. Most importantly, by studying the binding of iBodies to both, the inhibited and the uninhibited form of PSMA, we show the possibility to develop iBodies, which are able to recognize different conformations of the protein. The product is thus a highly soluble, conformation-specific antibody mimetic with nanomolar affinities, which are several orders of magnitude higher than in the case of the unconjugated peptide binders.

This method represents a proof-of-concept that can theoretically be applied to any target of interest. The possibility to create a stable, fully synthetic antibody mimetic with conformation-selective properties, could potentially contribute to more effective drug discovery and design.

Keywords: PSMA, antibody mimetic, phage display, conformation-selective binders.

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## B182 / Trogocytosis of Qa-1–restricted T cell receptors into tumor cells validates tumor recognition

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The prognosis of cancer patients has recently been improved by utilizing novel immunotherapies that inhibit the regulatory immune system. We have shown that down-regulation of the Helios transcription factor induces CD4+ regulatory T cell (Treg)

conversion into effector cells leading to enhanced anti-tumor immune responses(1). Helios is also the canonical transcription factor in CD8+ (CD44+CD122+Ly49+) regulatory T cells that have Qa-1 restriction for their antigen recognition. We have identified a series of T cell receptors that are specific for self-antigen FAM49B(p190-198)/Qa-1 within the CD8+ Treg subset and have utilized FAM49B(p190-198)-specific CD8+ Treg to provoke anti-tumor immune responses. Although FAM49B(p190-198) is a self-peptide that is normally degenerated in endoplasmic reticulum, it is stably presented in EL4 lymphoma cells. We found that EL4 subcutaneous tumor growth was delayed following transfer of FAM49B(p190-198)-specific T cells in the context of Qa-1 restriction.

To further understand this phenomenon, we have recently employed a trogocytosis assay. Trogocytosis in T cells and target cells is a biological phenomenon that allows cells to exchange membrane and molecules during immune synapse formation. Trogocytosis of FAM49B(p190-198)-specific T cells and EL4 revealed direct evidence of this interaction which has opened a new direction to explore surrogate peptides.

FAM49B(p190-198)/Qa-1 specific CD8 Treg were detected by FAM49B(p190-198)/Qa-1 tetramers after immunization with FAM49B(p190-198) peptide–loaded dendritic cells in CD8 Treg. T cell receptor cDNAs were amplified from sorted single cells and inserted into retroviral expression vectors for transduction in 58α−β− hybridoma cells. EL4-Qa-1 KO cells were generated using CRISPR/Cas9 technology. First, we cocultured TCR+ 58α−β− with EL4-WT or -Qa-1 KO loaded with cognate peptide for 2 hours and observed transferred TCR on EL4 cells. Next, to detect endogenous FAM49B(p190-198) presentation, TCR+ 58α−β− was cocultured with EL4-WT or -Qa-1 KO without peptide-loading followed by analysis of target cells. To identify more effective peptides than native FAM49B(p190-198) to induce potent immune responses, we synthesized various peptides substituting amino acids of the native peptide and tested them in the trogocytosis assay.

In this peptide loading system, we detected TCR transfer into EL4 WT cells both from OTI-TCR hybridoma and FAM49B-TCR hybridoma in the presence of cognate peptides, whereas we found TCR on EL4-Qa-1 KO cells only from OTI hybridoma but not FAM49B-TCR hybridoma with cognate peptides. Opposite transfer of some molecules (Qa-1 and TCR Vβ12) from EL4 WT into FAM49B-TCR hybridomas was also observed. Without peptide loading, TCR transfer became apparent after 12 hours and showed an increase of up to 8% of EL4-WT as positive for TCR after 20 hours while TCR+ EL4-Qa-1 KO were less than 1%. We also tested the variant peptides with different Qa-1 binding capacity in peptide-loading system and detected the same level of TCR transfer. Our data show that FAM49B-TCR recognizes endogenous FAM49B(p190-198) expressed by tumor cells in the context of Qa-1. Trogocytosis assay can also be utilized to identify potent surrogate peptides that expand this novel Qa-1–restricted subset.

Keywords: Trogocytosis, Qa-1, T cell receptor, Treg.

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## B183 / Combination of chemotherapy and DNA vaccination against tumor associated antigens effectively induces anti-tumor immunity in pancreatic cancer

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Pancreatic Ductal Adenocarcinoma (PDA) is one of the most lethal cancer, both for lack of effective screening method and resistance to chemotherapy (CT). Immunotherapy (IT) trials with immune check-point inhibitors did not achieve significant gain of survival yet. However, some CT agents, such as Gemcitabine (GEM), have several immune modulating effects and starting from the hypothesis that more immunogenic antigens can be induced by CT treatment, its ability to increase the susceptibility of PDA to IT was evaluated.

To discover tumor associated antigens (TAAs) suitable for IT, the serum of 28 PDA patients before and after 1 and 2 cycles of CT was profiled by serological proteome analysis (SERPA); the recognized antigens were identified by mass spectrometry and confirmed by ELISA. The proliferation, phenotype and cytokine production of T cells were evaluated on patients' PBMCs from the same cohort after in vitro stimulation with the recombinant proteins of the four most recognized TAAs (ENO1, G3P, K2C8 and FUBP1).

The number of TAAs recognized by circulating IgG in PDA patients' sera, as well as their ability to induce a complement dependent cytotoxicity against PDA cells, showed an increase after CT treatments. Moreover, for some of identified TAAs was observed a positive correlation between the increase of antibody titer after CT and a longer patients' survival.

Following stimulation of PBMCs with selected TAAs, was detected an increased T cell TAAs-specific proliferative response after CT. The evaluation of IFNγ/IL10 ratio, revealed that CT treatment shifted T cell TAAs-specific responses from regulatory to effector.

The role of CT to enhance the TAAs-specific adaptive response prompt us to exploit its effect in combination with the DNA vaccination. Genetically engineered mice that spontaneously develop PDA (KC) were treated with suboptimal dose of GEM prior of DNA vaccination against one of the selected TAAs, ENO1. Tumor lesions, immune infiltration and the titer of TAAs-specific antibody were evaluated. Splenocytes from KC mice were in vitro stimulated with selected TAAs and the production of IFNγ was analyzed. Of clinical relevance, KC mice treated with GEM prior of ENO1 DNA vaccination displayed smaller tumor lesions together with an increase of tumor-infiltrating CD4 and CD8 T cells, in comParison to mice vaccinated or GEM-treated only. Furthermore, CT increased specific antibodies and producing IFNγ T cells in vaccinated mice not only against ENO1, the TAA target of vaccination, but also against G3P, suggesting an antigen spreading effect of the combinatory treatment.

Overall these data indicate that in PDA CT strategy might be reconsidered, also with less toxic dosages, to enhance immune response against TAAs and renders them suitable targets for IT.

Keywords: Pancreatic cancer, DNA vaccination, Chemotherapy.

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## B184 / Splicing inhibition enhances the antitumor immune response through remodeling of the MHC I immunopeptidome

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Cancer immunoediting takes place in response to the cellular

immune response against tumor antigens. This dynamic process leads to the downregulation of the molecules associated with antigen production, processing and presentation and may explain the failure of many epitope-based immunotherapies in clinics. To counter the immunoselection of tumor antigens we highlight a new function for splicing inhibitors. They have been tested so far in clinics for their cytotoxicity directed toward cancer cells with defective splicing machinery. However, S. Apcher suggested a new mechanism of action for the splicing inhibitor isoginkgetin (Apcher et al. 2013). At subtoxic doses, the compound was able to increase the presentation of the non-conventional Pioneer Translation Products (PTPs). Those PTPs are polypeptides produced during a pioneer round of translation taking place in the nuclear compartment on pre-mRNAs, before the introns are spliced out. PTP-derived antigens can thus derive from coding sequences but also allegedly non coding sequences such as introns, 5' and 3' UTR regions or intron-exon junctions (Apcher et al. 2011). We showed in-vitro that isoginkgetin and its water-soluble and safe derivative IP2 increase the presentation of intron-derived epitopes at the surface of a variety of murine and human cancer cells. We further assessed IP2 activity in-vivo on MCA205 sarcoma and B16F10 melanoma tumors. We observed that IP2 slows down the growth of both models and that mice which experience complete tumor regression gain a long-term protection against further challenge with cancer cells. Nude mice and CD8+ T cell-depleted mice do not benefit from the treatment with IP2 suggesting that its action is mediated by the immune system. We confirmed that the compound is safe and does not exert a cytotoxic activity against cancer cells or healthy tissues. We then highlighted the alterations induced by IP2 in the MHC I immunopeptidome of cancer cells. We eluted and sequenced the peptides presented in H-2Kb and H-2Db molecules at the surface of mouse MCA205 sarcoma cells and mouse B16F10 melanoma cells. The proteomic analysis revealed that cancer cells display at their surface peptides from coding sequences but also from retained introns. We confirmed a result recently published by Smart, Margolis and colleagues: retained introns are a source of peptides for the MHC I presentation pathway (Smart et al. 2018). We identified peptides enriched at the surface of cancer cells upon treatment with IP2 and a new repertoire of peptides, missing from untreated cells. Among those IP2-specific peptides we found peptides already described in the Immune Epitope Database but also peptides derived from both coding sequences and retained introns that have never been identified before. Promising peptides were selected for further study in-vivo. We identified by ELISPOT IFNɣ reactive CD8+ T cells against IP2-induced peptides. The combination of IP2 treatment with peptide vaccination was effective in impairing the growth of MCA205 cells and improving survival. To sum up, we propose to use splicing inhibitors as immunomodulatory agents to reshape the repertoire of peptides presented at the surface of cancer cells. MS analyses allow the detection of newly presented antigens and the selection of immunogenic ones for further use in peptide-drug combinations. Splicing inhibition enhances the presentation of non-conventional antigens from allegedly non-coding sequences that proved to be relevant to induce an effective antitumor immune response.

Keywords: Tumor antigens, Introns, Spliceosome, MHC I immunopeptidome.

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## B185 / Discovery of tumor-associated antigens for triple-negative cancer immunotherapy

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The antigenic landscape of tumors is different from that of healthy tissue. The immune system is trained to probe these deviations from 'normal' and launch a robust cytotoxic response to eradicate malignant growth. Tumor rejection as the result of immune system activation greatly relies on the recognition of tumor-associated antigens and mutated antigens, known as neoantigens, presented as MHC-associated peptides on the cell surface. Despite the general realization of the neoepitopes role as major determinant for successful immunotherapy the most potent tumor antigens amenable for therapeutic application are currently unknown for many tumor types. This limits the rational design of effective and safe immunotherapies, especially for tumors with 'lower' mutational burden.

To discover novel tumor antigens for a tumor with relatively low mutational load we used triple-negative breast cancer as our tumor model. Triple negative breast cancer (TNBC) remains a challenging breast tumor subtype to treat with its aggressive nature and lack of therapy targets contributing to poor clinical outcome. Currently, TNBC consists of 10-20% of breast cancer cases and more likely affects younger persons. TNBC is considered an immunogenic subtype of breast cancer. PD-L1 expression levels are high and coincided with high rates of tumor infiltrating CD8+ T-cells suggesting tumor infiltrating lymphocytes contribute to tumor immunosurveillance (1, 2, 3). A recent clinical study further showed clinical benefit of anti-PD-L1 therapy in combination with paclitaxel in patients with metastatic TNBC (4). These data suggest immunotherapy, such as immune checkpoint inhibitors, are a promising treatment strategy for TNBC.

To further develop immunotherapy for TNBC, knowledge on the tumor infiltrating lymphocyte targets, and in particular on the MHC-I presented CD8+ T-cell epitopes, is required for both tumor associated antigens and neoantigens. Here we analysed the immunopeptidome, the collection of peptides associated to MHC-I, of triple-negative breast cancer. We employd direct detection of MHC-I presented peptides using MHC-I immunoaffinity purification combined with mass spectrometric identification of the MHC-I associated peptides. Bioinformatics analyses were employed to rank the tumor-associatead antigens for further in vivo analyses. We studied the anti-tumorigenic potential of selected tumor-associated antigens using a mouse model of TNBC and immunization by peptide vaccination. Immunogenicity of the peptides was tested in vivo following peptide vaccination and detection of interferon-gamma responses. Finally, we investigated the in vivo tumor control potential of 21 tumor antigens and their elicited T-cell responses in a mouse model. Our results show the potential of utilizing therapeutic cancer vaccination in the management of breast cancer and warrants further studies for identification of both shared and personalized tumor antigens for the cancer type.

Keywords: tumor antigen, triple-negative breast cancer, therapeutic cancer vaccination.

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## B186 / IDeATIon: Integrated Discovery of new immuno-molecular Actionable biomarkers for Tumors with Immune-suppressed environment

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The recent emergence of precision therapies targeting tumor mutations or immune molecules requires defining or better characterizing mutational patterns and the tumors immune environment that are none or insufficiently explored so far. The main research efforts focused on the genotyping of the most frequent tumors and allowed understanding mechanisms of oncogenicity and tumor evasion, or even lead to major progress in the targeting and development of new drugs. Current precision therapies either use immune checkpoint inhibitors which are highly effective in cancers with high tumor mutational burden in immunologically competent individuals, or target cancer molecular biomarkers defined either on tumors or on liquid biopsies from circulating tumor DNA and circulating tumor cells. In contrast, mutational profiling of tumors occurring in immune-suppressed individuals or environments for which the number continues to grow, have been poorly or not analyzed until now with these new molecular Methods. Indeed, relative to the general population, tumor frequencies, particularly lymphomas and lung cancers, increase significantly in transplant patients with immunosuppressive treatments or in people living with HIV (PLWHIV) even when they are under effective antiretroviral therapy. On the other hand, the lack of immune cells and immuno-surveillance observed in immune sanctuaries, such as the brain, could favor the emergence of a tumor, particularly lymphomas or gliomas. In these cases, the mutational tumor profile should differ from those observed in immune competent individuals, since the lack or alteration of immune functions should limit the control of tumor variants, or because other oncogenic events could be involved in these contexts. The number and immunogenicity of variants detected in a tumor may therefore differ for a given tumor between immune-competent and immune-suppressed individuals or in immune sanctuaries.

In this context the IDeATIon project from Pitié-Salpêtrière Hospital, Sorbonne-University Foundation, Sorbonne University and Inserm aims at discovering novel invasive and noninvasive immunomolecular biomarkers in rare but severe tumors occurring in immune-suppressed patients or immunological sanctuaries, compared to similar tumors in immune-competent patients. The highly innovative translational research IDeATIon program includes 200 patients with non-Hodgkin lymphomas or lung adenocarcinomas from the INCa national reference centers for PLWHIV (CancerVIH), transplant recipients (k-virogref), or primary brain tumors from the LOC and POLA reference centers. The program is testing the hypothesis that lack of immune environment and/or responses should lead to different tumor mutational profiles from similar tumors observed in immuno-competent individuals or tissues expressing immunity by analyzing the tumoral exomes and scores of neo-epitopes as well as analyzing circulating tumor DNA and cells in liquid biopsies. Analyses are in progress and results expected for 2020-21 will lead to:

-Identify novel invasive and non-invasive biomarkers for predicting and evaluating efficacy of future personalized and immune-based therapies,

-Compare tumor mutational profiles from immune-suppressed and immune-competent hosts,

-Discover hot spots of tumoral mutations, as mechanisms of tumor resistance and new molecular targets for future molecular therapeutic strategies,

-Define the tumor ImmunoMutanome as a score of neo-epitopes and detect neo-epitope (or mutation)-specific circulating T lymphocytes predicting tumor immunogenicity, disease outcome and potential response to immune-based therapies,

-Detect non-invasive tumoral biomarkers from liquid biopsies facilitate future diagnosis and monitoring of such tumors, -Identify biomarkers of tumor escape or resistance to treatments.

The expected results will be key to define more efficient future therapeutic strategies in these severe tumors occurring in immune-suppressed individuals or enviro

Keywords: Tumor environments, Immune-suppressed, Biomarkers, ImmunoMutanome.

References:
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## B187 / HPV-dependent cancer-testis antigen methylation and expression patterns in HNSCC

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Because of the restricted pattern of expression in normal tissues and expression in tumors cells, cancer-testis antigens (CTA) can be used as therapeutic targets or biomarkers. Their expression is almost exclusive for testis in healthy tissues, but they are expressed in different types of cancer. CTA expression is epigenetically regulated, most often through DNA methylation (Scanlan et al. 2004). Demethylating agents such as Decitabine (DAC) have been shown to increase CTA expression, which has been implied to improve CTA-specific immunotherapy (Siebenkas et al. 2017). HPV+ head and neck squamous cell carcinoma (HNSCC) displays a significantly different phenotype then it's HPV- counterpart. Because of that, we aimed to investigate the different CTA methylation and expression patterns in HPV+ compared to HPV- HNSCC tumors, as well as the effect of DAC treatment on the methylation and expression patterns.

DNA and RNA were isolated from HNSCC cell lines (3 HPV+ and 3 HPV- cell lines), and from fresh frozen tumor tissue samples of 25 oropharyngeal squamous cell carcinoma patients and subsequently used for Illumina EPIC Methylation assay and Illumina TruSeq stranded mRNA sequencing. HPV positivity was confirmed through HPV PCR and p16 immunohistochemical expression. HNSCC cell lines were treated with 2 QM DAC for 5 consecutive days. Statistical data analysis was preformed using Qlucore Omics Explorer. Principal component analysis and hierarchical clustering revealed specific patterns of cancer-testis antigen gene expression of HPV+ versus HPV- HNSCC tumors, where we identified a list of HPV-specific CTA candidate genes. HPV-specific patterns were also found in DNA methylation data, although not overlapping with gene expression patterns. The same clustering was confirmed in HNSCC cell lines. DAC treatment of the cell lines changed the methylation and expression patterns of CTA dramatically, resulting in strong demethylation and an increase in gene expression.

Expression and methylation of CTA genes differ between HPV+ and HPV- HNSCC. We showed the mirroring of tumor methylation and expression patterns in the cell lines in an HPV-dependent manner which justifies the usage of HNSCC cell lines as models. A specific correlation of gene methylation and expression could hardly be established. DAC treatment of cell lines influenced the methylation and expression patterns strongly, reducing the difference between the HPV-specific patterns of most of the CTA. HPV-specific CTA expression could result in different immunogenicity, whereas the DAC treatment possibly leads to an increased immunogenicity of HNSCC in general.

Keywords: Cancer-testis antigens, HPV, Methylation, Decitabine.

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tion machinery in colon and ovarian cancer cells. PLoS ONE. 2017; 12(6).

## B188 / Identification of mutant EGFR derived peptides that can bind stably to HLA-A*11:01

Amit Jain (NCCS), Jackwee Lim (Singapore Immunology Network, A*STAR).

Incurable EGFR mutant lung cancer is treated with tyrosine kinase inhibitors and chemotherapy drugs with dramatic improvements seen in survival outcomes over the past decade. While responses with immune checkpoint inhibitors have been dismal thus far, there is emerging data that immune responses directed towards mutant EGFR proteins exist and can be augmented with peptide vaccines. However, current tools to bioinformatically inform suitable peptides have inherent limitations in the context of HLA classes more prevalent in Asia including HLA-A*11:01. Hence, we sought to ascertain more direct unbiased biophysical evidence of MHC-peptide binding in order to be able to identify suitable EGFR peptides for use as vaccines. As a pilot study, the binding of two peptides derived from wildtype EGFR (HVKITDFGL) and L858R mutant EGFR (HVKITDFGR) was evaluated with the ProImmune REVEAL Rate Assay. HVKITDFGR was noted to form a stable complex with HLA-A*11:01 while the wild type peptide did not. We then embarked on assessing 160 peptides that included all possible 8- to 12-mers across 4 clinically relevant EGFR mutations, L858R, T790M, C797S, and one specific Exon 19 deletion, with two independent assays that directly measure peptide-MHC-I complex stability using HLA-A*11:01. Peptides that were predicted with NetMHCpan4.0 to be strong binders (top 2%) including KITDFGRAK (L858R) were generally highly ranked in both assays. However, several peptides predicted to be only moderate binders had high peptide-MHC-I complex stability. For example, SLLDYVREH (C797S) is predicted to have only moderate binding to HLA-A*11:01 but on our assay was noted to form peptide-MHC complex that had stability comparable to KITDFGRAK as well as an EBV control peptide known to be a strong binder. Of note, strong binding predicted for several peptides on NetMHCpan4.0 was found to be inconsistent with their respective peptide MHC-I complex stability in our assays. Hence, we discovered that firstly, most peptides had a good correlation between their NetMHCpan4.0 predicted peptide-MHC binding and peptide-MHC complex stability; secondly, some peptides predicted to be moderate binders were nevertheless able to form stable peptide-MHC complexes; and thirdly some predicted strong binders did not form stable MHC-peptide complexes. This suggests that bioinformatically predicted binding affinity alone is inadequate to predict peptide immunogenicity and that direct measurement of peptide MHC stability is feasible and can additionally inform peptide choice for cancer peptide vaccine strategies.

Keywords: MHC-peptide stability.

## B190 / Intron retention as a source of neoantigens arising from somatic splice site mutations

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Mutations which are typically annotated as silent or non-coding can still cause significant changes to protein sequence through modification of splice signals. We have observed evidence of intron retention in clinical samples when a mutation disrupts the nucleotides before or after an exon boundary, especially when the germline sequence differs from the canonical "AG|GT" 5' splicing signal. If such retained introns are translated they can generate large stretches of novel amino acids, potentially creating tumor-specific neoantigens. To assess the frequency of productive intron retention events arising from splice site mutations, we used a computational tool called Isovar [1] to examine the RNA sequencing data of 14 patients from the PGV001 neoantigen vaccine trial [2]. We found that ~0.1% of expressed somatic mutations in coding regions contain retained introns. These mutation specific intron retention events were not found in RNA reads lacking the mutant allele. Many of the detected novel sequences contained peptides which were predicted to be strong binders of patient HLA alleles.

Keywords: neoantigens, intron retention, splicing.

References:
[1] https://github.com/openvax/isovar

## B191 / PrDx a novel stability-based MHC-epitope prediction tool applied for selection of 40 neo-epitopes in a colorectal cancer patient

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Mutations in cancer cells can lead to the formation of neo-epitopes presented by Major Histocompatibility Complex (MHC) class I or II. These neo-epitopes may be recognized by CD8+ or CD4+ T cells and trigger an immune response. Since only a small fraction of the neo-epitopes will be displayed by the MHC class I or II, one of the major challenges of neo-epitope vaccination is to predict which neo-epitopes will be presented by MHC and prone to elicit a T cell response. Software tools such as netMHC, MHCFlury and others tend to be over predictive, since the majority of the data used to train these Methods are based on affinity assays. Several studies have indicated that peptide MHC stability assays may be a better predictor of immunogenicity instead. We have performed stability assays on a number of MHC I and II alleles and used these data to train PrDx, that relies on a combination of different machine learning Methods such as; random forest, feed forward neural networks and recurrent neural networks. We have shown how PrDx can be used for a more precise identification of true T cell epitopes in a patient case with colorectal cancer. Immunitrack's NeoScreen platform was applied in this first patient case. Out of 20, 000 mutations identified by whole exome sequencing the top 100 predicted epitopes to 8 of the patient's MHC alleles was analysed by in vitro stability assays (800 peptides in total) to select 40 of the most promising neo-epitope vaccine candidates. Where after the patient was treated with the vaccine in spring 2019. Training in silico prediction tools on high quality stability data will provide selection of more immunogenic neo-epitopes and thereby pave the way to effective cancer vaccine design.

Keywords: Novel prediction tool, MHC, Neo-epitopes, Cancer vaccine.

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## B192 / A pan-HLA predictor of neoantigen presentation to the tumor cell surface and T cell reactivity

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Although HLA binding algorithms predict binding affinity of a peptide to a limited number of HLA alleles reasonably well, they do not predict processing and presentation to the cell surface (i.e., the immunopeptidome). In fact, only 15%–20% of predicted HLA-peptide binders are presented to the cell surface, and are therefore potentially reactive to T cells. Erroneous predictions may be addressed with time-consuming and laborious experiments, such as mass-spectrometry (MS). However, in silico predictions have now proven to be very useful in prioritizing therapeutically relevant immunogenic peptides (1). Previous in silico studies that predict naturally processed and presented peptides to the cell surface, have focused on only one of the many steps in the antigen processing and presentation pathway (such as TAP transport or proteasome cleavage, etc). Additionally, previous antigen processing prediction tools have been trained and are therefore applicable to a limited number of well studied HLA alleles, making it challenging to make predictions for not so well-characterized alleles. Here, we outline a series of updates on our machine learning approach trained on MS elution data that predicts, in a pan-HLA manner, natural processing and presentation of neoantigens to the cell surface. The predictor is integrated with multiple immune parameters to predict neoantigens for any HLA allele for

both Class I and Class II. Our recent updates in the latest release of our neoantigen prediction technology (described in this presentation) primarily focus on diverse and comprehensive proprietary data, and new models to T cell reactivity to cell-surface presented neoantigens. By analysing previously published clinical data, we illustrate its application leads to a significantly improved identification of neoantigen targets for personalized cancer immunotherapy.

Keywords: neoantigen, tumor antigen, bioinformatics, personalized vaccines.

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# Vaccination strategies

## B193 / The efficacy of the autologous tumor vaccine with adjuvant in vivo gene electrotransfer of plasmid DNA encoding IL-12 and local tumor irradiation varies between tumor models

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Although all relevant antigens through which the immune system can mount a response are present in tumor cells, during oncogenesis tumor cells evolve to avoid the immune system. Whole tumor cell vaccines are comprised of modified tumor cells inactivated by irradiation and aim to enhance the recognition of the tumor and the activation of the immune system. In clinical studies such vaccines, GVAX and Algenpantucel-L, use virally transduced tumor cells irradiated with high doses of radiation to prevent further mitosis [1]. However, these cells remain viable at the time of vaccination. In our study, we aimed to develop and test a safer autologous tumor vaccine comprising of tumor cells killed by irradiation and plasmid DNA encoding interleukin 12 (IL-12). To prepare our vaccine, cells were first exposed to an irradiation regime of 3 fractions of 5 Gy to evoke an immunogenic effect of radiation on cells. After two days a lethal dose of 30 Gy followed and 1×106 of non-viable tumor cells were mixed with 50 Qg of plasmid DNA encoding IL-12 to make up the autologous tumor vaccine. In vivo studies on the B16-F10 and CT26 tumor models in syngeneic 6 – 8 week old mice were performed to test our hypothesis of a synergistic effect of the vaccination and local tumor irradiation. The vaccination was performed distantly from the tumor and it comprised of a subcutaneous injection of the autologous tumor vaccine and in vivo gene electrotransfer (GET) of plasmid DNA encoding IL-12 contained in the applied vaccine [2]. GET was performed using a contact hexagonal multielectrode array (MEA) in vivo. The MEA electrode with a central pin was positioned to encompass the applied vaccine and 24 electric pulses were applied [3]. Additionally, mice were treated with concurrent local tumor irradiation with a dose of 15 Gy. Post-treatment, tumor volumes were calculated from three orthogonal diameters of the tumor, which were measured every second day using a Vernier Caliper. All experimental procedures were performed in accordance with the EU directive (2010/63/EU) and with the guidelines of the Ministry of Agriculture, Forestry, and Food of the Republic of Slovenia (permission no. U34401-1/2015/16). The results varied based on the tumor model. We observed a synergistic effect in the B16-F10 tumor model, where mice receiving vaccination and local tumor irradiation had a significantly delayed tumor growth compared to mice receiving only vaccination or only local tumor irradiation ($P < 0.05$). On the other hand, no synergistic effect of vaccination and local tumor irradiation was observed in the CT26 tumor model. In this model, the only significant difference in tumor growth was observed between mice which received local tumor irradiation and mice which did not ($P < 0.05$). The results suggest a greater contribution of the vaccination to local tumor irradiation in a less immunogenic tumor model, i.e. B16-F10, than in an immunogenic tumor model, i.e. CT26; however, further studies are required to support this statement.

Keywords: tumor vaccine, gene electrotransfer, interleukin 12, ionizing radiation.

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## B194 / Polyfunctional CD4+ T cells are efficiently induced by a live influenza virus vaccine but not by inactivated virus

Michael J. Hogan (Children's Hospital of Philadelphia), Wenzhi Song (Children's Hospital of Philadelphia), Laurence C. Eisenlohr (Children's Hospital of Philadelphia).

The classical model of CD4+ T cell activation holds that antigens are mostly presented on MHC class II (MHCII) via an exogenous route, i.e. originating from outside the antigen-presenting cell. In contrast, our previous work (Miller et al., Nat. Med. 2015) in the influenza virus (flu) model shows that CD4+ T cells are activated far more efficiently when MHCII-associated antigen originates from endogenous sources, as is typical in a viral infection. Specifically, we found that a live flu vaccine elicits a greater frequency and breadth of interferon gamma-producing CD4+ T cells in mice than does a killed flu vaccine. Here, we sought to more fully characterize the differences in CD4+ T cell functionality induced by live vs. killed virus vaccines.

C57Bl/6 mice were immunized with live influenza virus strain A/Puerto Rico/8/1934 or a large excess dose of inactivated virus. Efforts were made to control for both total antigen load and adjuvant effects. Flu-specific CD4+ T cells were evaluated by peptide/MHCII tetramer staining, intracellular cytokine staining after peptide restimulation, and flow cytometry. Total and flu-specific T follicular helper (Tfh) cells were also quantified by co-staining for canonical Tfh markers in combination with tetramers.

Greater frequencies of flu-specific CD4+ T cells were elicited by live vs. killed virus, as measured by tetramer staining or expression of the cytokines interferon gamma, tumor necrosis factor, or interleukin-2. Of particular note, live virus induced a significantly higher frequency of polyfunctional CD4+ T cells, producing two or more cytokines simultaneously when stimulated with peptide. Furthermore, the percentage of the flu-specific CD4+ T cells that was polyfunctional was higher for live vs. killed virus, suggesting that the type or quality of CD4+ T cell differentiation was specifically modulated by the vaccine format. This difference in polyfunctional CD4+ T cells was also observed when an antigenically distinct virus was mixed with killed flu as an adjuvant or when killed flu was administered in a prime/boost series. Studies are ongoing to investigate the effect of vaccine format on Tfh cell differentiation.

The data are consistent with a model whereby endogenous antigen presentation on MHCII transmits more potent activating signals to CD4+ T cells than exogenous presentation, perhaps due to differences in peptide/MHCII complex density and kinetics. This leads to a greater level of polyfunctional type 1 cytokine production, which has been associated with enhanced control of chronic infections and tumor clearance. These results may therefore be relevant to the design of more effective vaccines and immune therapies for infectious diseases and cancer. Michael Hogan is a Cancer Research Institute Irvington Fellow supported by the Cancer Research Institute.

Keywords: Vaccine, CD4 T cells, MHC class II.

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Miller MA, Ganesan AP V., Luckashenak N, Mendonca M, Eisenlohr LC. Endogenous antigen processing drives the primary CD4+ T cell response to influenza. Nat Med. 2015;21(10):1216-1222.

## B195 / The Bivalent DNA Vaccine against Human Adenovirus Serotype 4 and 7

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Adenovirus(AdV) is one of the ubiquitous pathogens which causes respiratory illness. Adenoviral hexon protein is trimeric in nature, which can induce conformational neutralizing antibodies and late viral regulatory protein, L4-100k, plays an important role in the trimeric formation. Currently, there are no commercial drugs and vaccines against AdV infection on the market.

Here, we used pVAX1, FDA-approved DNA vaccine vector, as the expression vector and connected hexon and its corresponding L4-100K with the linker IRES to construct the target plasmid pvax1-hu4-IRES-100k and pvax1-hu7-IRES-100k. It was verified by western blot that AdV-4 and AdV-7 hexon had a high expression in 293T. We immunized Balb/c mice with the monovalent DNA vaccine (pvax1-hu4-IRES-100k or pvax1-hu7-IRES-100k) and bivalent DNA vaccine (pvax1-hu4-IRES-100k and pvax1-hu7-IRES-100k) in a dose dependent manner by electroporation (EP) 3 times at week 0, 2 and 4. Sera were obtained at 2, 4, 6, 8, 10 and 12 weeks. The anti-hexon humoral immune responses were analyzed by ELISA and neutralization assay. The results showed that monovalent AdV-4 and bivalent AdV-4/7 hexon specific binding antibody were detected in both 50μg and 100 μg DNA vaccine regimen at week 4 sustaining to week 12. However, the monovalent AdV-7 and bivalent AdV-4/7 hexon specific binding antibody was detected small amount at week 6 with 100 μg DNA vaccine. In addition, the AdV-4 neutralizing antibody titer is higher than AdV-7. The former studies showed that the immunogenicity of AdV-4 hexon is stronger than that of AdV-7 hexon. So we are trying to optimize the AdV-7 hexon sequence to increase the immunogenicity.

Next we will infect the immunized mice with AdV4 106 pfu or AdV7 2×106 pfu . The lungs will be stained by HE to analyze histopathological variations and viral titration will be detected by RT-PCR. Since there is no animal model of adenovirus in the world, we are exploring this aspect to conveniently prove the effectiveness of DNA vaccine.

Keywords: Hexon, Adenovirus, DNA vaccine.

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## B196 / A Universal Booster Vaccine for Chimeric Antigen Receptor T cells

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Chimeric antigen receptor (CAR) T cell therapy has shown dramatic clinical responses in hematologic malignancies, with a high proportion of durable complete remissions elicited in leukemia and lymphomas. However, achieving the full promise of CAR T therapy, especially in solid tumors, will require further advances in this form of cellular therapy. A key challenge is maintaining a sufficient pool of functional CAR T cells in vivo. We recently developed a strategy to target vaccines to lymph nodes, by linking peptide antigens to albumin-binding phospholipid-polymers. Constitutive trafficking of albumin from blood to lymph makes it

ideal chaperone to concentrate these "amphiphile-vaccine" molecules in lymph nodes that would otherwise be rapidly dispersed in the bloodstream following parenteral injection. These lipid-polymer conjugates also exhibit the property that they insert in cell membranes on arrival in lymph nodes. Here, we generated amphiphile CAR T ligand (amph-ligand) vaccine by exploiting these dual lymph node targeting and membrane-decorating properties to repeatedly expand and rejuvenate CAR T directly in vivo- in native lymph nodes. We evaluated this approach in the presence of a complete host immune system. Amph-ligand vaccine effectively trafficked to lymph nodes, triggered massive CAR T expansion, increased donor cell polyfunctionality, and enhanced anti-tumor efficacy in multiple immunocompetent murine solid tumor models. We demonstrate two approaches to generalize this strategy to any CAR, enabling this simple non-HLA-restricted approach to enhance CAR T functionality to be applied to existing CAR T cell designs. In summary, we have developed a universal booster vaccine to enhance CAR T cell activity against solid tumors.

Keywords: Vaccine, CAR T, Amphiphile.

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## B197 / An immunogenic neoantigen vaccine for glioblastoma designed with machine learning-based algorithms

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Glioblastoma (GBM) is a largely incurable manifestation of cancer cells in the brain with an extremely poor clinical outcome. Novel strategies are needed to improve patient survival, quality of life, and overall outcomes. Low GBM mutation rates suggest it may be challenging to identify novel tumor-specific epitopes capable of priming CD8+ T cells. Despite this hurdle, we present here a proof-of-concept experiment showcasing the design of a GBM neoantigen-based vaccine using state-of-the-art computational approaches and the testing of its immunogenicity in the orthotopic GL261 mouse model.

The GL261 cell line and normal Blk/6 spleen tissue underwent whole exome sequencing to identify somatic single nucleotide variants. Tumor cell RNA was also sequenced to evaluate transcript expression. A total of 421 expressed missense mutations were screened using Ancer, an innovative neoantigen screening platform that combines machine learning-based MHC I and MHC II neo-epitope identification with removal of inhibitory regulatory T cell epitopes for optimal personalized cancer vaccine design. One hundred and sixty-six neoantigens were ranked according to predicted immunogenicity, clonality, and expression. The 18 highest ranking sequences were synthesized as peptides and co-formulated with poly-ICLC or poly-ICLC + Montanide for immunization of GL261 tumor-bearing animals beginning four days after intracranial tumor implantation. Vaccine immunogenicity was evaluated at days 11, 18, and 25 post-implantation in LAMP1 (CD107A) degranulation and IFNg secretion assays.

Ex vivo peptide-stimulated splenocytes and maxillary/mandibular lymphocytes showed increased cell surface expression of LAMP1 in CD3+CD8+ T cells from GL261-bearing animals that were treated with peptide + poly-ICLC as early as day 11. LAMP1 expression reached 82% of CD8+ T cells on day 11 and decreased to 33% by days 18 and 25 despite booster doses. Thirty-two percent of CD8+ T cells expressed surface LAMP1 in animals that were vaccinated with peptides + poly-ICLC + Montanide at day 11, which receded to 23% at day 18 and to baseline by day 25. Degranulation was primarily vaccine-specific as control animals exhibited baseline LAMP1, with the exception of day 11 where 22% of CD8+ T cells expressed LAMP1 in animals vaccinated with poly-ICLC alone.

IFNg secretion was observed by ELISpot assay in immune cells from peptide + poly-ICLC treated animals at day 18 (210 SFC/10e6 cells above Background) and sustained at day 25 (159 SFC/10e6 cells above Background). Similar IFNg levels were detected in animals that received the Montanide formulation at days 18 and 25, although IFNg secretion was detected as early as day 11 (75 SFC/10e6 cells above Background), unlike the peptide + poly-ICLC formulation. Control animals did not respond to ex vivo peptide stimulation. In silico screening of neoantigen sequences using specialized tools offers the possibility of enriching and designing new vaccines with higher quality candidates. Additionally, these techniques allow for a dramatic reduction in turnaround times from several weeks, typically observed for ex vivo-based screenings, down to minutes, while not compromising vaccine immunogenicity as showcased here in an orthotopic GBM tumor model. Follow-up studies include assessment of T cell infiltration in brain tissues collected from immunized mice and the impact of the Ancer-GBM vaccine on animal survival.

Keywords: glioblastoma, personalized cancer vaccine, neo-epitope, GL261.

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## B198 / Final results of first-in-human phase I study of INVAC-1, an optimized human telomerase (hTERT) DNA vaccine in patients with advanced solid tumors

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INVAC-1 is an optimized DNA plasmid encoding an inactive form of human Telomerase Reverse Transcriptase (hTERT), a universal tumor antigen expressed in most of human tumors with little or no expression in normal somatic cells. Primary pharmacodynamics, safety pharmacology and toxicology studies, including biodistribution and local tolerance in animal models, showed that INVAC-1 was enzymatically inactive, immunogenically safe and well tolerated. In murine models, we demonstrated that INVAC-1 was able to induce hTERT specific cellular immune responses with CD4+ Th1 effector and memory CD8+ T-cells. In a HLA-A2 spontaneous mouse sarcoma Sarc-T2r model, INVAC-1 vaccination was able to slow tumor growth and increased survival rate by 50% in tumor-bearing mice (1). We report here the final results of a First-In-Human (FIH) study, Phase I, open label, 3+3 escalation design and multiple dose study examining the safety and tolerability of INVAC-1 in patients with relapsed or solid refractory tumors.

This two center Phase I trial evaluated INVAC-1 given monthly for a minimum of 3 cycles and up to 9 cycles by intradermal injection followed by electroporation (n=20) or using a needle-free injection system (n=6). Primary objectives included safety, tolerability and dose limiting toxicities to identify the maximum tolerated dose and recommended phase 2 dose. Secondary objectives included immune response and anti-tumor activity. Immune monitoring included detection of autoantibodies, inflammatory cytokine levels in blood as well as circulating cell subpopulation phenotyping and

CD4/CD8 anti-hTERT immune responses assessed by IFN-γ ELISpot. Anti-tumor activity was evaluated through RECIST 1.1 adapted to immune response.

26 patients with refractory/progressive tumors were enrolled and treated at 3 escalating doses of 100, 400 and 800 Qg. Overall, the majority of patients (58%, 15 patients) experienced disease stabilization according to RECIST. For 11 of them, the treatment was extended, up to 9 cycles. INVAC-1 was safe and well tolerated with no dose-limiting toxicities. No significant biological signs of autoimmunity were observed. No significant modification in inflammatory plasma cytokines levels was observed after INVAC-1 administration. INVAC-1 was shown to trigger cytotoxic CD8 T cell response as well as Th1-polarized anti hTERT CD4 T cell response in 63% of patients. This specific anti-hTERT immune response was enhanced by adding anti-PD-1 immune checkpoint inhibitor nivolumab ex vivo. Moreover, preliminary ex vivo data suggested that INVAC-1 vaccination was able to promote epitope spreading. Finally, a non-significant trend towards improved Overall Survival was observed in INVAC-1 immune responders: 17.4 months vs. 7 months for responders vs. non-responders, respectively.

results indicate that INVAC-1 was safe and well tolerated at the doses and schedule tested. Disease stabilization was achieved for the majority of patients (58%) during the treatment period and beyond, which is comparable to early clinical findings for anti-immune checkpoint monoclonal antibody trials. Moreover, INVAC-1 vaccination induced a robust hTERT specific Th1 CD4 immune response as well as cytotoxic CD8 T cell response. Finally, INVAC-1 immune responders showed a non-significant trend to prolonged OS compared to non-responders. Phase II study of INVAC-1 in hematologic malignancies has been initiated.

Keywords: telomerase, vaccine, phase 1.

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## B199 / Oral therapeutic peptide vaccines reduce tumour growth in an orthotopic mouse model of colorectal cancer

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Orally administered vaccines that stimulate local mucosal immunity could be useful for the treatment of colorectal cancer. To test therapeutic effect of oral vaccines for colorectal cancer treatment, subunit vaccines containing the immunodominant CD8+ epitope (AH1) from the CT26 colorectal carcinoma cell line, a chimeric tetanus-diphtheria toxoid CD4+ T cell helper peptide and the TLR2 ligand Pam2Cys were formulated into oral vaccines. Since vaccines administered orally must be protected from gastric hydrolysis, two types of lipid-based carriers were used, liposomes and W/O/W double emulsions. The immunological and therapeutic effects of the oral vaccine were evaluated in naïve mice and in an orthotopic mouse model of colorectal cancer, where intestinal CT26 tumours were established through injection of the cells into the wall of the caecum. We demonstrated that oral vaccination with lipid-encapsulated peptide subunit vaccines was able to slow tumour growth and induce lymphocytes infiltration into tumours. Immune cell analysis revealed that both oral subunit vaccines stimulated local and systemic immune responses, however in most cases only responses to emulsion vaccines were statistically significant. Oral vaccination induced systemic T cell response in the spleen and accumulation of T and B lymphocytes, and CD11c+F4/80+CD11b+ innate immune cells in mesenteric lymph nodes. Overall, the study supports the hypothesis that orally delivered therapeutic cancer vaccines are able to generate local and systemic anti-tumour immune responses and slow the growth of colorectal cancer cells.

Keywords: oral, vaccine, colorectal cancer.

## B200 / LSP-based therapeutic vaccine impact on tumor angiogenesis

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Therapeutic antitumor vaccination is engineered to stimulate specific T-cell responses against the tumor. However, very few vaccination strategies have demonstrated their therapeutic efficacy in clinic. The improvement of such vaccination approaches is therefore required. The choice of the tumor antigen and the vaccine design remains essential criteria to consider (1).

In this context, we have studied the therapeutic efficacy of a long synthetic peptide vaccine containing CD4 and CD8 epitopes of the inhibitor of apoptosis survivin (SVX vaccine). Survivin is overexpressed in most cancers and its expression is associated with a poor prognosis, a resistance to chemotherapies, the development of metastases and the induction of tumor angiogenesis (2).

We recently demonstrated the efficacy of SVX vaccine in mice associated with a tumor growth delay, 30% of complete tumor regression and an increased survival in different cancer models. This efficacy was associated with the induction of specific CD8 T cells responses, multifunctional CD4 T cell responses and the establishment of CD8 memory responses (3).

In order to face the challenges of the poor therapeutic efficacy of vaccine as monotherapy in advanced cancers, knowing that vaccination strategies do not appear to allow overcoming immunosuppression induced by the tumor microenvironment, we are studying the optimization of the SVX vaccine in combination with other immunomodulatory approaches such as antiangiogenic treatments.

The rational of combining therapeutic vaccination and antiangiogenic treatments have been demonstrated in mice but results diverge in term of administration scheduling between the two therapies (4).

Because survivin promotes tumor angiogenesis by its action on the expression of VEGF (5), we are firstly investigating the impact of SVX vaccination on tumor angiogenesis to have a better understanding of mechanisms involved in the angiogenic and immune remodeling induced by each of the therapies individually.

To do so, we are studying the impact of SVX vaccination on different angiogenesis markers such as VEGF production by the tumor, tumor hypoxia, vessel number and vessel normalization within the tumor and during tumor development. In this context, we showed that SVX vaccine is associated with a decreased VEGF production following the vaccination, at early days of tumor development and with an increase in pericyte coverage in vaccinated mice.

By further understanding the impact of the vaccine on tumor angiogenesis, we are hoping to determine the best scheduling strategy to fully optimize vaccine efficacy by its combination with antiangiogenic treatments in order to limit tumor escape.

Keywords: therapeutic vaccine, survivin, tumor angiogenesis, immunosuppression.

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## B201 / Antigens released by Salmonella-infected tumor cells as a novel vaccine platform

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Advancement in cancer immunotherapy has revealed the importance of targeting neoantigens: tumor specific antigens that prompt a strong antitumor response escaping from the central T tolerance. We have previously shown that infection of melanoma cells with Salmonella (SL) induces the up-regulation of membrane hemichannels and the transfer of antigens between adjacent cells (1). Now we demonstrate that SL not only leads to the transfer of immunogenic antigens between adjacent cells but also the release of peptides in the extracellular milieu. We tested the released peptides as components of a vaccine formulation given to prevent tumor progression in a murine model of melanoma and we demonstrated that are immunogenic. We attested that also human melanoma cells infected with SL release peptides; moreover, peptide release is hemichannel-dependent as it is completely blocked by heptanol, a hemichannel blocker. A mass spectrometry analysis of peptides released by infected human melanoma cells revealed that among them there are known tumor antigens, novel tumor epitopes and potential neoantigens. The novel antigens selected as strong HLA binder by predictive in silico tools were synthesized and their antigenicity further confirmed. They indeed primed healthy donor peripheral blood mononuclear cells and induced the expansion of CD8-T cells that specifically killed tumor cells in vitro. Encouraging results have been obtained with the therapeutic treatment of pet dogs with spontaneous osteosarcoma and high grade sarcoma with a vaccine formulation based on peptides released by their own tumor cells following SL infection. Moreover, dog patients that underwent a heterologous setting of vaccination with peptides released by tumor cells of a dog suffering of the same tumor type have shown improvement in the survival thus indicating that peptides released upon Salmonella infection are shared among tumor cells of different patients. We propose the treatment of patients-derived tumor cells with Salmonella as a strategy to obtain potent immunogenic antigens that could be applied in clinical studies as a vaccine formulation.

Keywords: Vaccine, Tumor antigens, Immunotherapy.

References:
1 Saccheri et al, Sci Transl Med. 2010

## B202 / Development of a cancer vaccine strategy by tumor antigen targeting to CD169+ antigen presenting cells.

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CD169-expressing macrophages are present in lymphoid organs at the site of antigen entrance to capture pathogens. Previously, we have shown that these CD169+ macrophages efficiently transfer antigen to cross-presenting dendritic cells and B cells for the induction of strong CD8+ T cell and humoral responses, respectively. Our aim is to develop a cancer vaccine strategy by means of tumor antigen targeting to CD169+ macrophages and we have investigated two approaches.

First, using anti-CD169 antibody-antigen conjugates we show that both ovalbumin (OVA) peptide as well as protein antigen targeting to CD169 results in strong primary and recall CD8+ T cell responses and protective immunity against B16-OVA tumor outgrowth in mice. Likewise, Trp-2 and gp100 melanoma peptides conjugated to anti-CD169 antibodies stimulated strong CD8+ T cell responses in wild type mice and human MART-1 peptide-CD169 complexes stimulated CD8+ T cell responses in HLA-A2.1 transgenic mouse. Finally, human CD169-expressing monocyte-derived dendritic cells cross-presented human gp100-CD169 conjugates to gp100-specific CD8+ T cells.

Secondly, we have investigated liposomes containing CD169-binding ganglioside ligands to target antigens to CD169+ antigen presenting cells in mice and man. We show that these liposomes efficiently bind to CD169+ macrophages in mice and result in T and B cell responses against OVA present in the liposome. In addition, human CD169+ expressing monocyte-derived dendritic cells take up CD169 ligand- and tumor antigen-containing liposomes and cross-present tumor antigen to CD8+ T cells.

In conclusion, different approaches to target tumor antigens to CD169+ antigen presenting cells demonstrate a strong capacity to stimulate immune responses and should be further explored as a vaccination strategy for cancer.

Keywords: targeting, dendritic cell, CD169+ macrophage, vaccine.

## B203 / Carbamylation of lysine residues mediated by myeloid derived suppressor cells in the tumor environment make excellent targets for CD4 T cell mediated cancer immunotherapy

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Stress induced post-translational modifications (siPTM) stimulate T cell responses that result in anti-tumor immunity. One such siPTM is homocitrulline, which is produced by the chemical reaction of isocyanic acid with lysine. Three homocitrullinated peptides, one from vimentin and two from aldolase A induced potent IFNγ responses in HLA-DR4, DR1 or DP4 transgenic mice. Responses were CD4 mediated and specific to the homocitrullinated peptides. In vitro assays showed that healthy donors and cancer patients have an oligoclonal CD4 T cell repertoire that recognizes at least one of these homocitrullinated peptides. Vaccination with the homocitrullinated peptides 4 days after implant with HLA matched B16F1 tumor increased survival in HHDII/DP4 (90%, $p<0.0001$), HLA-DR4 (70%, $p=0.0042$) and HHDII/DR1 (50%, $p<0.0001$) transgenic mice. Tumor therapy was dependent upon the presence of CD4 cells as it was abrogated with CD4 depletion but not CD8 depletion. Indeed, direct CD4 killing was crucial as only tumors expressing class II were rejected. Isocyanic acid is generated from thiocyanate by myeloperoxidase (MPO). MPO is expressed by neutrophils and also in a population of tumor associated myeloid derived suppressor cells (MDSCs). We show that in vitro generated MDSCs are able to drive homocitrullination of tumour cell proteins. Antibody depletion of MDSCs in tumor bearing mice, attenuated the anti-tumor effect of the homocitrullinated peptide vaccines ($p=0.048$). Therefore we propose that MDSC-produced MPO catalyzes the build-up of isocyanic acid which diffuses into the tumor cells and homocitrullinates cytoplasmic proteins which are then degraded and presented by MHC-II. Together this data proves that MDSC-mediated homocitrullination of tumor proteins can be directly targeted by vaccine induced CD4 cells and provides a wide-reaching target for cancer vaccines.

Keywords: Homocitrulline, Aldolase, Myeloid derived suppressor cells.

## B205 / DNA immunization against tumor epitopes using a versatile delivery platform that exploits inherent mechanisms of antiviral defense

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Cancer vaccines have many advantages such as exquisite specificity, low toxicity, and the potential for a durable treatment effect. However, the limited immunogenicity of tumor antigens makes them challenging to develop and novel immunization strategies are required for a proper activation of T cells. In particular, strategies to increase the immunogenicity of cancer DNA vaccines are needed for moving toward clinical applications [1, 2].

We hypothesized that plasmids encoding engineered viral proteins could efficiently stimulate the immune system which is able to detect and eliminate certain viral threats.

We showed that, when electroporated in the muscle of mice, a plasmid coding for the vesicular stomatitis virus glycoprotein (VSV-G) induced locally increased levels of IL-6, IL-12 and CCL2 suggesting its ability to trigger innate immunity. We then demonstrated that the VSV-G sequence can be engineered to incorporate various foreign T-cell epitopes. When inserted in permissive sites of the VSV-G sequence, both MHC-I and MHC-II ovalbumin-restricted epitopes were adequately processed and induced the proliferation of specific CD8+ and CD4+ T cells, respectively. Antigen specific in vivo killing assay showed that 65% of target cell killing was obtained when these two epitopes were co-delivered and a significant therapeutic effect against B16F10-OVA tumor was observed. The therapeutic efficacy was further reinforced by the co-administration of anti-PD1 and anti-CTLA4 antibodies. Several engineered VSV-G sequences were constructed to target various tumor epitopes and they significantly delayed tumor growth in multiple preclinical models: B16F10 melanoma, P815 mastocytoma and CT26 colon carcinoma, demonstrating the versatility of the delivery platform.

Plasmids encoding modified VSV-G sequences have the many advantages of DNA vaccines that are simple to construct, easy to produce and clinically applicable. They are thus promising candidates as tumor epitope delivery systems for future clinical developments.

Keywords: DNA vaccine, Delivery system, Cancer vaccine, Plasmid.

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## B206 / Gangliosides-containing liposomes bind to human CD169+ antigen presenting cells and stimulate tumor antigen-specific T cells

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Pancreatic cancer forms a major cause of cancer related deaths with a very short mean overall survival of just 6-12 months and new therapies are urgently needed. While single immune-checkpoint inhibitors have proven to be ineffective due to the relatively low-mutational rate, the addition of tumor vaccines may augment anti-tumor immune responses. Our previous work has already demonstrated that CD169+ macrophages can stimulate superior immune responses in mouse models [1]. Using liposomes containing CD169-binding gangliosides, we hypothesized that these liposomes could be used to target and to deliver pancreatic cancer antigens to human CD169+ antigen-presenting-cells (APCs) and to drive pancreatic antigen-specific immune responses.

We generated liposomes containing different gangliosides GM3, GD3, GM1, GD1a, GT1b, with DiD tracer, and toll-like receptor ligands (TLR) MPLA. We included Wilms' Tumor 1 (WT1) peptide in the liposomes as this antigen is overexpressed pancreatic cancer. Binding at 4°C and uptake at 37°C were assessed with different APCs: THP1 cell line overexpressing CD169, human monocyte-derived dendritic cells (moDCs) and macrophages (moMacs), human peripheral blood mononuclear cell (PBMCs), and human splenic macrophages using (imaging) flow cytometry. Using blocking antibody against CD169, we determined the specificity of this binding. For antigen presentation assays, moDCs were pulsed with WT1 or gp100 peptide-containing ganglioside liposomes and subsequently co-cultured with WT1- or gp100-specific CD8 T cell clones.

We observed that liposomes containing gangliosides efficiently bound to and were taken up by CD169-overexpressing THP1 cells, human moDCs and moMacs, and human splenic macrophages. moDC and moMac liposome binding and uptake could be further enhanced by IFNα-induced CD169 upregulation, and was blocked by αCD169 antibody. Unsupervised high dimensionality reduction viSNE analysis showed that ganglioside-containing liposomes were specifically taken up by human peripheral blood CD169+ monocytes and CD169+ DCs. Incorporation of TLR ligand MPLA into ganglioside-liposomes induced cytokine production by CD169+ moDCs. Importantly, moDCs exposed to gangliosides-liposomes containing pancreatic cancer antigen WT1 peptide stimulated WT1-specific CD8 T cell activation. On the same line, ganglioside liposomes containing melanoma-associated antigen gp100 were taken up and cross-presented to gp100-specific CD8 T cells. Our ongoing experiments will evaluate the intracellular trafficking of ganglioside-containing liposomes using imaging flow cytometry.

To conclude, several ganglioside-containing liposomes could be used to target and to deliver tumor-associated antigens to human CD169+ APCs and to activate T cells. Future studies will focus on whether these liposomes can be used to induce pancreatic tumor antigen-specific T cell responses in vivo.

Keywords: CD169, Antigen-presenting cells, Ganglioside-Liposome, Pancreatic cancer.

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## B207 / Development of personalized cancer nanovaccines against neoantigens

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Cancer immunotherapy has made unprecedented progress in the last few decades. In particular, checkpoint blockade and adoptive T cell therapy have shown remarkable clinical results. However, the clinical efficacy of therapeutic cancer vaccines remains modest. One of the foot causes is the generally weak and short-lived antigen-specific T cell responses due to inefficient delivery of antigen/adjuvant to the secondary lymphoid organs and lack of effective cross-presentation. Recent studies provided solid evidence that cancer vaccines against tumor neoantigens—immunogenic peptides resulting from somatic mutations—can be immunologically and therapeutically effective in human patients. Here we developed a novel nanovaccine platform for synthetic long peptides (SLPs) to target neoantigens in order to enhance the efficacy and safety of cancer vaccines. The SLP nanovaccines were prepared through reversible chemical crosslinking of antigen and adjuvant components to form a nanogel (NG) with controlled size and responsiveness for antigen/adjuvant release. The NG with the optimized size targeted lymph nodes (LNs) and dendritic cells (DCs) effectively in vivo. The responsiveness of chemical linkers was designed to facilitate the intracellular release and subsequent cytosol delivery of SLPs for enhanced cross-priming of cytotoxic T cells. The SLP-NG vaccines elicited 3.5-fold higher frequencies of antigen-specific cytotoxic T cells than Montanide, one of the most potent vaccine adjuvants used in clinical trials.

In conclusion, the SLP-NG provided a promising platform for the development of personalized neoantigen-based cancer vaccines.

Keywords: vaccine delivery, neoantigen, nanovaccine, personalized vaccine.

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## B208 / Lipo-based vaccines as an approach to target dendritic cells for induction of strong anti-tumor responses

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Effective vaccination strategies in cancer patients for the elimination of the tumor require the induction of a strong tumor specific immune response that activates players of the innate as well as the adaptive immune system. Both innate and adaptive immunity is driven by antigen presenting cells (APC). Therefore, human skin resident APCs such as dermal dendritic cells (dDCs) and Langerhans cell (LCs) are an attractive target for in situ anti-tumor vaccination. Delivery of cancer vaccines to DCs can be addressed by targeting of receptors like C-type lectin receptors, such as DC-SIGN and Langerin, by use of their natural ligands, so called glycans as for example Lewis Y (Ley). In an ex vivo human skin explant model we have previously shown that modification of melanoma tumor antigens with glycans greatly facilitates endosomal routing and cross-presentation of melanoma tumor antigens by targeting C-type lectin receptors on human dDCs and LCs. dDCs can also activate invariant natural killer T (iNKT) cells, which operate at the boundary of innate and adaptive immunity, through presentation of αgalactosylceramide (αGalCer) in a CD1d dependent manner. Indeed, we have shown before that injected αGalCer is taken up by skin APCs and results in activation of iNKT while simultaneously enhancing the efficacy of a tumor vaccine.

In this study, we used liposomes as a platform to develop a melanoma cancer vaccine containing palmitoylated synthetic long peptides (SLP) of MHC class I and II epitopes of the melanoma antigen gp100 and αGalCer to target dendritic cells for activation of both innate and adaptive players of the immune system. Combination of lipo-tumor specific antigens gp100 and αGalCer in one lipo-formulation resulted in strong gp100 specific CD8+ T-cell activation and increased IFNγ secretion by iNKT. Moreover, incorporation of Ley in the lipid bilayer of the liposomes for C-type lectin targeting increased not only targeting and uptake by moDCs and dDCs but also enhanced gp100 specific CD8+ T cell and iNKT activation in both in vitro assays and an ex vivo human skin model. Additionally, we determined the capacity of DCs loaded with liposomes to prime human primary CD8b+ T-cells and could detect superior activation of T-cells after loading of DCs with the Ley modified liposomes. We conclude that the combination of a targeting moiety and SLP together with αGalCer in one vaccine formulation induces strong anti-cancer innate and adaptive immunity and could therefore be a new potential vaccination strategy for cancer patients.

Currently this cancer vaccine is tested in an in-vivo mouse model systems to eliminate B16 melanoma in a therapeutic setting and in combination with checkpoint inhibition.

Keywords: DC-targetin, vaccine, iNKT, liposomes.

## B209 / Pattern recognition receptor expression and maturation profile of dendritic cell subtypes in human tonsils and lymph nodes

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Dendritic cells (DCs) with a capacity of antigen cross-presentation are of key interest for immunotherapy against cancer as these cells can induce antigen-specific cytotoxic T lymphocyte (CTL) responses. This study describes the frequencies of DC subtypes in human tonsils and lymph nodes, and their phenotypic aspects that may be targeted by adjuvant measures. From human tonsils and neck lymph nodes, DCs were identified through flow cytometry, and subset frequencies of CD123+ plasmacytoid DCs (pDCs) as well as CD11c+ myeloid DCs (mDCs) were investigated. Their maturity statuses were assessed, and selected surface receptors were explored focusing on such that may promote CTL responses, including C-type lectin receptors (CLR), Toll-like receptors (TLR), and chemokine receptor XCR1. (1, 2)

PDCs as well as CD1c+, CD141+, and CD1c-CD141- mDCs were detected in tonsils and lymph nodes. Both sites featured a similar presence of DC subsets, with pDCs being dominant and CD141+ mDCs least frequent. All DC subtypes featured a low degree of maturation based on CD80 and CD86 expression. Expression of pattern recognition receptors (PRRs) CD206, CD207, DC-SIGN, TLR2, TLR4, and the chemokine receptor XCR1 indicated DC subset-specific receptor profiles. CD1c+ mDCs selectively presented high levels of CD206 and CD207. Significantly higher levels of TLR4 were observed for DC subsets in lymph nodes c.f. tonsils.

We conclude that tonsils and lymph nodes share common features in terms of DC subset frequency and maturation as well as PRR and XCR1 expression pattern. Our work suggests the possibility that tonsils as well as lymph nodes may be viewed as vac-

cine deposition sites in DC-mediated immunotherapy.

Keywords: Dendritic cell, Lymph node, tonsil, PRR.

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## B210 / Dendritic cell vaccination sensitizes established pancreatic carcinoma to CD40-agonistic antibody treatment

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Although immunotherapy yields striking results in various malignancies, results in pancreatic cancer have been disappointing. Both a highly immunosuppressive tumor microenvironment and a dense desmoplastic stroma have been found to preventing immunotherapy efficacy by prohibition of proper T-cell infiltration in these tumors. In an aggressive murine pancreatic ductal adenocarcinoma model resembling human pancreatic cancer, we assessed the efficacy of vaccination with tumor cell lysate-loaded dendritic cells (DC) in combination with agonistic CD40 targeting. DC vaccination and anti-CD40 therapy are known to induce effector T cells and promote involution of tumor stroma, respectively. As acquiring autologous pancreatic tumor for lysate generation is often unsuccessful, we also investigated the use of an allogeneic tumor lysate sharing tumor antigens with pancreatic cancer which could enable an off-the-shelf strategy and standardize treatment across patients.

Immune competent C57BL/6 mice were subcutaneously inoculated with pancreatic tumor cells (KPC3). Mice with established tumors were vaccinated with DCs and consequently treated with CD40 agonistic antibodies (FGK45). DCs were generated with a GM-CSF bone marrow culture and stimulated overnight with CpG along with either pancreatic (KPC3) or mesothelioma (AE17) tumor lysate. Tumor progression was monitored and immune responses in various tissues were analyzed using multicolor flow cytometry and NanoString nCounter mRNA expression analysis.

Vaccination increased systemic CD4+ and CD8+ T cells and delayed tumor growth accompanied by increased tumor infiltrating lymphocyte (TIL) numbers. Tumor growth was significantly delayed in mice treated with mesothelioma-lysate DCs compared to mice treated with DCs in the absence of tumor lysate. In vitro, the frequencies of TNFa+, IFNg+, CD107a+ and granzyme B+ expressing CD8+ T cells of mesothelioma-lysate DC treated mice were increased upon stimulation with pancreatic cancer cells compared to untreated or mice treated with DCs without tumor lysate, confirming tumor-reactivity of the vaccine-induced T cells. In an advanced disease model, DC vaccination combined with anti-CD40 significantly delayed tumor growth and improved survival whereas both monotherapies were not effective. Interim blood analysis showed significant increases in frequencies of activated and proliferating T cells in treated animals, and those cells also displayed an effector memory phenotype. This was more pronounced for CD4+ T cells in mice treated with DCs while anti-CD40 therapy induced a confined response in CD8+ T cells. Combination therapy demonstrated this for both CD4+ and CD8+ T cells. Also, increased TIL numbers were found in both monotherapy and combination therapy treated mice. Extensive characterization of the tumor microenvironment revealed that although anti-CD40-treatment increased CD8+ TIL numbers, these tumors showed signs of exhaustion. Tumors of anti-CD40 treated mice had increased numbers of TILs expressing multiple co-inhibitory molecules (i.e. PD-1, TIM-3, VISTA, CD39 and NKG2A). The combination of DC therapy with anti-CD40 prevented this, thereby improving therapy efficacy.

These results demonstrate the potency of this novel form of combination immunotherapy and reveals a mechanistic insight into the requirements of effective immunotherapy in pancreatic cancer. In order to investigate whether combination immunotherapy is feasible, safe and potentially effective in pancreatic cancer patients, we recently launched a clinical trial with allogeneic mesothelioma lysate-DC vaccination for resected pancreatic cancer patients in an adjuvant setting (Dutch Trial Register NL7432).

Keywords: Pancreatic ductal adenocarcinoma, Tumor microenvironment, Desmoplastic stroma, T-cell exhaustion.

## B211 / Can metastases treat glioblastoma? a first in human study evaluating the safety and efficacy of vaccination with non-attenuated, live autologous glioblastoma cells

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Glioblastoma (GBM), which grows uninhibited in the brain, almost never metastasizes outside of it. The rare occurrence (<0.5%) of overt GBM extracranial metastasis are paradoxically associated with significantly enhanced survival of the metastasized patients.

We previously reported that the highly malignant F98 Fischer-rat GBM model, which grows aggressively in the brain, spontaneously regresses when injected subcutaneously as live unattenuated cells. The peripheral (subcutane) growth and spontaneous regression of live glioma cells markedly enhances the survival of all rats, and cures (survival >9 months) approximately 50% of rats challenged with F98 cells intracranially. The results in the F98 model were corroborated in the CNS1 astrocytoma model in Lewis rats. The fractions of cured rats in either model were the highest reported in published literature.

The spontaneous rejection of live autologous glioma cells inoculated in peripheral sites was immune-mediated. This location-biased immune response was termed 'Split Immunity' - a tumor that thrives in an immune-privileged site (e.g., the brain) may be inhibited by injecting live unmodified tumor cells in a site that is not privileged, generating protective immunity that spreads back to the privileged site. To evaluate the applicability of the 'Split Immunity' approach in humans, we ran a first in human (FIH) study. Two recurrent GBM patients were vaccinated subcutaneously, initially with their autologous irradiated tumor cells, then with autologous, non-attenuated, live tumor cells.

The treatment caused no serious adverse events. The injected live autologous tumor-derived cells did not grow at the subcutane inoculation site, nor did they systemically metastasize, as evaluated by choline 18F PET-CT. Standard blood tests showed no treatment-related adverse effects. Both patients' quality of life (QoL) questionnaires demonstrated subjective improvements in their self-evaluated "global health status".

The treatment had demonstrated several signs suggestive of efficacy. MRI evaluations in both patients using 'treatment response assessment maps' (TRAM) demonstrated strong increases in the 'treatment-response' component following live-cell vaccination, compatible with pseudo-progression-like responses.

Using elaborate multicolor flow cytometric panels, we detected potent tumor-cell specific polyfunctional activation in 2.5% and 5% of peripheral cytotoxic T cell (CTL) and T helper cells (Th), respectively, 3 days following live-cell vaccination.

Flow cytometric immune mapping of all tumoral immune cell subsets showed major changes from baseline that occurred shortly after live-cell vaccinations. We observed major increases in both patients, of all monitored intratumoral dendritic cell (DC) subsets. Patient 2's CD1c+ and CD141+ conventional DC and plasmacytoid DC had increased, together, from 1/62, 000 to 1/130 cells. Both his intratumoral CD141+ and CD1c+ conventional DC had upregulated their CD86 maturation marker. Lymphoid cells had also increased: Patient-1 showed the highest frequency of intratumoral CD56brt natural killer (NK) cells from all previously fully mapped brain tumor patients. Patient-2's intratumoral CTL and Th had increased from the range of 1/500-1000 to 1/60-80 following treatment. The overall tumor immune infiltrate of Patient-2 following treatment exceeded that of 42 previously fully-mapped brain tumor patients. All flow cytometric data was corroborated using immunohistochemistry.

Patient-2 who was treated by a tighter vaccination protocol than Patient-1 had survived 19 months following GBM recurrence, outliving about 90% of other recurrent GBM patients.

In summary, the accumulated FIH results are suggestive of both safety and initial efficacy of the 'Split Immunity' approach. Based on the FIH results, we shall soon initiate a phase I/II clinical trial on 8 recurrent- and 4 newly-diagnosed GBM patients.

Keywords: clinical trial -first in human, Whole cell vaccine, Glioblastoma, Live non attenuated vaccine.

## B212 / Active immunotherapy induces long-lived CD8 T lymphocytes and protects against tumor recurrences in preclinical model

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Sexually transmitted HPV infects 8 out of 10 people worldwide. Persistent infections caused by the virus can lead to cancer mostly in the cervix and oropharyngeal region. Cervical cancer is considered the fourth most common cancer in women, accounting more than 500, 000 million cases/year and around 270, 000 deaths in the world. The conventional treatments (surgery, chemotherapy and radiotherapy) are costly, invasive and less effective, particularly for more advanced stages of the disease. In this context, active immunotherapeutic procedures based on the activation of specific T cell responses, which are capable to eliminate tumor cells, represent a better choice regarding the available treatments. Since 2004, researchers of the Laboratory of Vaccine Development (University of Sao Paulo, Brazil) have developed an innovative technology based on the genetic fusion of HSV-1 glycoprotein D (gD) and the HPV-16 E7 protein in two different technological platforms: a DNA vaccine (pgDE7h) and purified recombinant protein (gDE7). Our previous results demonstrated that gD acts as an adjuvant leading to activation of mouse CD11c+ CD8a+ and human BDCA3+ dendritic cells (DC) specialized in antigen cross-presentation to CD8+ T cells. In the present study, the results were generated after administration of the antitumor active immunotherapies combined to cisplatin treatment – the main chemotherapy used in cervical cancer patients. C57BL/6 mice were previously transplanted in the vaginal region with TC-1 LUC tumor cells, which expresses luciferase and HPV-16 E6/E7 oncoproteins. Once the tumors were established, the animals received one dose of cisplatin (CIS) intravenously followed by two doses of immunotherapy: 100 micrograms/dose of pgDE7h intramuscularly associated with electroporation (DNA-EP) or 30 micrograms/dose of gDE7 subcutaneously mixed with poly I:C (PTN-pIC). The results revealed a synergism between cisplatin and the therapeutic vaccines leading to 100% of survival and full regression of tumor in 90% of mice for both formulations. In addition, the association of cisplatin with the immunotherapies promoted higher frequencies of E7-specific cytotoxic T lymphocytes (CTL) in the bloodstream and CD8+ T cells in the vagina, leading to full protection against intravaginal TC-1 re-challenge. Also, we tried to mimic tumor recurrence at a site other than the primary tumor site. For this purpose, surviving mice treated CIS/DNA-EP or CIS/PTN-pIC were re-challenged with TC-1 LUC injection in the tongue. As a result, 80% and 90% of mice treated with CIS combined to DNA-EP or PTN-pIC, respectively, showed tumor rejection with increased numbers of CTL in the bloodstream, effector memory T cells in the spleen and tissue resident memory CD8+ T cells in the tongue. Our results confirm the synergism of this association and unveil a new therapeutic approach against HPV-associated tumors.

Keywords: immunotherapeutic, vaccines, HPV.

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## B213 / Oral administration of melatonin potentiates the immunotherapeutic efficiency of a DNA vaccine against HPV-16-associated tumors

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Human Papillomavirus (HPV) is the most common sexually transmitted infection in the world. Practically all cases of cervical cancer, as well as an increasing proportion of anogenital, head and neck cancer, are associated with HPV. Although improved knowledge of both cancer immunology and the molecular pathogenesis of HPV-associated tumors, patients with advanced cancer have a low clinical outcome. Indeed, the metabolic plasticity of the tumor environment frequently influences the efficacy of therapeutic procedures, including those based on immunological tools. Therefore, the identification of efficient molecules able to control tumor progress represents an important opportunity for new therapeutic strategies, particularly in combination with standard-of-care treatments. In this concern, melatonin has been evaluated in clinical trials as an immunometabolic adjuvant to improve cancer treatment, since this indole exhibited a myriad of in vitro oncostatic effects. In the present study, we evaluated the therapeutic potential of a novel immunotherapy approach focusing on the combination of melatonin with a DNA vaccine (pgDE7h) produced from the genetic fusion of the HPV-16 E7-oncoprotein with the envelope glycoprotein (gD) of herpes simplex virus (HSV). As a tumor cell model, we used the TC-1 cells, a murine tumor cell line encoding the HPV-16 E6 and E7 oncoproteins. In vitro, we observed that melatonin was able to inhibit the adhesion, migration, and proliferation of TC-1 cells, being these phenomena related to the induction of cell cycle arrest in the G0/G1 phase. Interestingly, in a mouse grafted TC-1 tumor model, the combination therapy of melatonin with pgDE7h not only increased survival but was also able to lead to total tumor regression in 65% of the animals. The antitumor effects of this combination approach were associated with the in vivo proliferation and activation of CD8+ T cells, together with increased migration of dendritic cells into the tumor microenvironment, as well as the decrease of suppressor myeloid cells. Taken together, our results highlight the use of melatonin as an immunometabolic adjuvant to achieve a robust antitumor immune response, representing a new and promising alternative for improving the efficacy of immunotherapeutic treatments of HPV-associated tumors. Financial Support: FAPESP

Keywords: Human Papillomavirus, Melatonin, DNA vaccine, Cancer immunotherapy.

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## B214 / Dendritic cell targeting of human papillomavirus-Associated Tumor vaccine by means of anti-DEC205 mAb

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Cervical cancer represents a serious public health problem in the world and corresponds to one of the greatest causes of tumor-related deaths in women. Human Papillomavirus (HPV HPV-16 and 18 are the most common virus genotypes related to cancer induction and cause more than 50 and 35% of the cervical cancer cases, respectively. Despite the availability preventive and therapeutic Methods, high mortality rate is still a challenge for advanced forms of the disease. The development of safe and effective therapeutic anti-tumor vaccines is a potential alternative for reducing the death tools associated with the disease. Dendritic cells (DCs) are professional antigen-presenting cells that initiate and direct immune responses mediated by T cells. DEC205+ CD8a+ dendritic cells, specifically, are considered the most clinically relevant for initiating CD8+ T cell responses critical for killing tumors. Therefore, mAb-mediated targeting of antigens to surface receptors on these cells may improve the performance of anti-tumor vaccines leading to enhanced immunogenicity of antigens in vivo. In this study, we used the anti-DEC205 mAb fused to HPV16 E7 oncoprotein and evaluated the impact in the control of Human Papillomavirus-Associated Tumors. For this purpose, the recombinat αDEC-E7 mAb was produced in HEK293T cells and purified from culture supernatant. In functional assays, mAb was able to bind transgenic CHO cells that constitutively express the murine DEC205 receptor and DCs of mouse spleen. To study the therapeutic potential of this strategy, we adopt a preclinical model based on the transplantation of TC-1 and TC-1 luc tumor cell lineages, which express HPV-16 E6 and E7 oncoproteins. C57BL/6 mice, previously transplanted with the tumor cells, were inoculated subcutaneously twice with the αDEC-E7 mAb in the presence of poly(I:C). The therapeutic properties of the strategy were evaluated in tumors implanted either intravaginally and subcutaneously. We demonstrated that in both models, the use of the αDEC-E7 was capable to therapeutically control the growth of the tumor cells and to induce activation of E7-specific IFN-+-producing CD8+ T cells. Our results demonstrate that the antigen DC-targeting approach represent a promising strategy in the fight against tumors induced by HPV.

Keywords: Dendritic cell, DEC205, HPV, CANCER.

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## B215 / Developing and implementing a generalizable, straightforward Method to measure antigen presentation

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The ability to directly measure presentation of specific MHC-associated antigens could facilitate new avenues of investigation, including screening strategies to boost the presentation of vaccine antigens. Current techniques to assess presentation either require reporter T cells or antibodies, and are therefore poorly generalizable between antigens, or use a mass spectrometry (MS)-based readout. MS-based Methods focus on characterizing the global set of MHC-presented peptides, rather than a specific antigen(s) of interest, and require large numbers of cells (10^7-10^9). We developed a protocol that is straightforward, quantitative, and higher throughput than existing techniques. By drawing on Methods that employ direct elution from the surface of intact cells using a mild acidic buffer and combining this with targeted, high-sensitivity LC-MS/MS analysis, we were able to observe and quantify sub-femtomole levels of an MHC-presented antigen. Using a model antigen, we examined the time- and concentration-dependence of antigen presentation. We verified that this technique is generalizable to different antigen sequences and cell types, including primary murine antigen presenting cells. With this technique in hand, we then began to explore the impact of D-amino acid substitution on antigen presentation. Future work will determine whether single D-amino acid substitutions within the MHC-binding epitope sequence will boost the level of antigen presentation by favorably altering proteolytic stability. The assay we have developed enables us to screen this and other approaches to enhance MHC-associated antigen presentation.

Keywords: antigen presentation, vaccine.

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## B216 / The role of HOCl in improving dendritic cell-based cancer vaccines

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Thanks to the critical role of dendritic cells (DCs) in activating cytotoxic T-cell responses, DC-based vaccines constitute a great therapeutic opportunity for cancer immunotherapy. Despite this, clinical trials generally displayed a very limited therapeutic efficacy whilst maintaining a very low toxic profile. Previous work demonstrated that treating cancer cells with hypochlorous acid (HOCl) greatly increases their immunogenic potential, improving downstream responses both in vitro, in vivo and in cancer patients treated with DCs pulsed with HOCl-treated antigen sources, compared to the untreated counterparts. Despite these interesting results, the mechanisms behind are still elusive.

To address this question, we conducted a proteomic analysis based on LC-MS/MS to map and characterize modifications introduced by HOCl treatment using a human melanoma cell line (A375) as a model system. As expected, we observed a very large increase in the oxidation of several amino acids and of several proteins upon HOCl treatment, compared to the untreated counterpart. However, these modifications were not completely random but focused instead on specific classes of amino acids (e.g. Met and Lys among others) and on a specific subset of proteins. Furthermore, through gene ontology enrichment analysis we were able to map specific cellular pathways affected by the incubation with HOCl, giving a complete picture of both the molecular and biological consequences of HOCl treatment of cancer cells (e.g. cell cycle arrest, metabolic reprogramming, among others). Based on this, we subsequently pulsed human DCs using A375 cells treated with HOCl (Ox-DCs) or left untreated (ctr-DCs) as antigen source and then analyzed the set of peptides presented on both MHC-I and MHC-class II complexes, through affinity purification followed by LC-MS/MS. results showed a great increase in MHC-class II antigen presentation in Ox-DCs compared to ctr-DCs. In addition to this, we also identified ~2, 000 peptides uniquely pre-

sented on MHC-II complexes in Ox-DCs and not detected in ctr-DCs. Instead, concerning MHC-I complexes, levels and identity of peptides were overall comparable between the Ox-DC and ctr-DC conditions. Based on these results we conclude that the observed increased immunogenicity of HOCl-treated antigen sources is likely due to an increased MHC-II dependent stimulation of the T cell compartment (i.e. CD4+ and T cell helper response) that further support tumor killing cytotoxic immune responses (e.g. CD8+ T cells, NK cells). Future work will focus on characterizing the function and phenotype of downstream, stimulated CD4+ T cells and verify this hypothesis.

These results further contribute to the development of more effective and immunogenic DC-based vaccines and to the molecular understanding of the mechanism behind the increase immunogenicity induced by HOCl.

Keywords: dendritic cell, tumor antigens, vaccine, proteomics.

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## B217 / Depletion of myeloid derived suppressor cells (MDSC) but not regulatory T cells (Treg) by DCF chemotherapy correlates with a good prognosis in anal squamous cell carcinoma (SCCA) patients

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Purpose: SCCA is a rare disease accounting for 2.6% of gastrointestinal malignancies and it is associated with E6 and E7 oncoproteins encoded by HPV16 in 90% of cases and the incidence rate of this cancer is increasing each year worldwide. Our research group has shown that the addition of Docetaxel to 5-Fluorouracil plus Cisplatine (DCF) induces in a high percentage a long-term complete remission in retrospective and prospective clinical trials; Epitopes-HPV01 and 02. Notwithstanding these results, the impact of adaptive immune responses and the role of MDSC and Treg cells were never investigated in this disease.

Experimental Design: Peripheral Blood mononuclear cells from Healthy donors (n=19, EFS Besancon) and SCCA patients included in Epitopes-HPV02 study (Before (n=64) and after (=48) DCF chemotherapy) were monitored. The presence of T-cell responses after a short term T-cell assay against HPV16 E6/E7 derived-peptivator and hTERT derived-peptides was measured by $IFN_{\gamma}$ ELISPOT assay. MDSC and Treg cells were characterized by flow cytometry.

Results: Peripheral Treg count was increased in SCCA patients compared to healthy donors. Thus, we decided to select 5% as a threshold to analyze Treg influence on clinical outcomes. Treg level does not significantly influence SCCA patient survival when measured before DCF (mPFS of 11.7 vs 13.67 months p=0.79) or after DCF chemotherapy (mPFS of 11.7 vs 16.57 months p=0.53). Peripheral MDSC count increased in SCCA patients compared to healthy donors. Thus, we decided to select 4% as a threshold for further MDSC analyses. The distribution of antigen specific T cell responses was higher and more diverse in SCCA patients with low-risk MDSC percentage. At baseline, patients with high levels of MDSC had a worse PFS (12.23 vs 13.13 months p=0.5). After DCF chemotherapy, we observed that patients with low MDSC counts had an improved PFS (7.6 vs 21.9 months p<0.0001).

Conclusion: Our results have demonstrated that a low level of MDSC cells is associated with a lower progression rate in SCCA patients; and was not influenced by antigen specific T-cell responses. These data show that MDSC cells are indeed a prognostic factor in SCCA patients after DCF chemotherapy.

Keywords: M-MDSC, biomarker, SSCA, DCF chemotherapy.

## B218 / Neoantigen specific CD4+ T cells boost local radiotherapy-mediated in situ vaccination

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Therapeutic RNA-based cancer vaccines have shown great promise in various pre-clinical and clinical trials driving cancer antigen-specific immune responses. The possibility of combining liposomal formulated, systemic RNA (RNA-LPX) vaccination with standard clinical care, such as local radiotherapy, is an attractive approach not yet assessed. Whereas RNA-LPX driven anti-tumor responses rely on priming of antigen-specific T-cells, radiotherapy acts on a multitude of different levels. Recent evidence suggests that local radiotherapy engages the immune system, leading to anti-tumoral innate and adaptive immune responses. Whereas the importance of cytotoxic CD8+ T cells has been well described, the role of CD4+ T cells in radio-immunotherapies is less clear. Here, we describe for the first time that CD4+ neoantigen RNA-LPX vaccination can potentiate radiotherapy-mediated T cell priming in murine neoplasia.

We conducted a preclincal study in the CT26 tumor model, performing neoantigen CD4+ RNA-LPX vaccination and local, 12 Gy, radiotherapy. Combination therapy treated mice display an increased tumor rejection and a survival benefit over radiotherapy treatment alone that is accompanied by a boost of gp70-specific CD8+ T cell in blood. In tumor re-challenge experiments, we furthermore observed immunological memory formation against CT26 tumors after combination therapy. We next tested the efficacy of combination therapy in CT26gp70ko tumors, lacking the immunodominant gp70 antigen. Here, interestingly, we observe tumor rejection in approximately half of the mice, indicating the relevance of other, still unknown, CD8+ T cell antigens in driving effective anti-tumoral responses. Combination therapy mediated effects are abrogated when CD8+ T cells are depleted, highlighting the relevance of cytotoxic CD8+ T cells in CT26 tumor rejection. We analyzed the phenotype of intratumoral CD8+ T cells and observed an increase of activatory and migratory molecules (CD44, CX3CR1, LFA-1) after radiation treatment. The combination with CD4+ RNA-LPX additionally increases the effector function of intratumoral CD8+ T cells, responding to gp70 peptide ex vivo restimulation to a higher magnitude. CD8+ TCR sequencing revealed that radiotherapy induces a more clonal response – meaning the expansion of single TCR clones within the tumor microenvironment. In summary, our findings indicate that high dose (12 Gy) local radiotherapy leads to transient delay of tumor growth and induction of tumor specific CD8+ T cell, which benefit from CD4+ neoantigen RNA-LPX vaccination, rendering their immune response more functional.

Keywords: Radiotherapy, CD4 neoantigens, RNA-based cancer vaccines.

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## B219 / A melanoma vaccine based on heat-shock conditioned tumor cell lysate inhibits tumor growth and increases mice survival, even in the absence of anti-PD1 treatment

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Clinical immunotherapeutic strategies using immune-checkpoint blockers (ICB), such as anti-CTLA4, anti-PD1 or anti-PD-L1 antibodies, have demonstrated durable survival benefits in patients with melanoma and other solid tumors. Nevertheless, an important percentage of these patients remain refractory, suggesting that its combination with active immunization may improve this response rate [1]. In this context, cancer vaccines become a complementary and attractive alternative for cancer treatment. Optimal delivery of antigens (Ags) and adequate use of adjuvants are crucial factors for vaccine success. Here, a prototype of a generic therapeutic vaccine for malignant melanoma treatment was tested in an experimental C57BL/6 murine melanoma preclinical model. This vaccine, named TRIMELVax is based on a heat shock-conditioned melanoma cell lysate (TRIMEL) combined with the Concholepas Concholepas hemocyanin (CCH) as a specific adjuvant [2]. Vaccination with TRIMELVax has demonstrated to activate immune responses against B16F10 tumors in vivo, in both prophylactic and therapeutic approaches, inhibiting tumor growth and prolonging animals' survival. These immune responses were associated with increased CD8+ T lymphocytes and dendritic cell tumor infiltration, in contrast to reduced neutrophils intratumor presence, measured by immunohistochemistry and multiparametric flow cytometry. Moreover, increased antibody production against tumors was detected by ELISA. Also, TRIMELVax delays tumor growth at comparable levels with anti-PD1 therapy. Remarkably, while anti-PD1 treatment doesn't increases survival of tumor bearing mice, TRIMELVax prolongs mice survival even in the absence of combinatorial therapy with anti-PD1 antidody [3]. As TRIMELVax seems to be a potent vaccine against melanoma, this encourages the testing of this therapy in future clinical trials.

Keywords: vaccines, melanoma, cell-lysate, heat-shock.

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## B220 / Pancreatic cancer therapy based on combination of DNA vaccination and PI3Kgamma inhibition

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Pancreatic ductal adenocarcinoma (PDA) is the fourth leading cause of cancer mortality in developed countries, with one of the poorest prognoses among all cancers. Although 10-15% of patients are candidates for gross total surgical resection, recurrence is frequent, and the overall 5-year survival rate is around 8%. Using a proteomic approach, we have identified alpha-Enolase 1 (ENO1) as PDA-associated antigens. We have shown that ENO1 DNA vaccination efficiently prolongs survival of engineered mice that spontaneously develop PDA (both KC and KPC mice). Targeting of Phosphoinositide-3- kinase (PI3K) gamma and delta isoforms is an effective way to inhibit the suppressive activity of immune-regulatory cells, thus strengthening the anticancer immune response. Recently, we have demonstrated that PI3K gamma play a critical role in PDA by driving the recruitment of myeloid derived suppressor cells into tumor tissues and it's genetic or pharmacologic inhibition effectively inhibits PDA progression and metastasis. In this study we assessed the hypothesis that the targeting of myeloid derived suppressor cells, via pharmacological PI3Kgamma inhibition, synergizes with ENO1 DNA vaccination by inducing a strong and sustained immune response.

KPC mice were vaccinated four times with ENO1 starting at 4 weeks of age; two weeks later mice were treated with the PI3K-gamma inhibitor TG100-115 (2, 5 mg/kg), for further two weeks. At sacrifice humoral and cellular responses were analyzed by histology, immunohistochemistry, enzyme-linked immunosorbent assays, flow cytometry, and enzyme-linked immunosorbent spot and cytotoxicity assays. Mice that received ENO1 and TG100-115 therapy showed a significant decrease in tumor size in comParison to both ENO1 alone (p=0.0385) and PBS (p=0.0003) treated mice. This correlated with a decrease of CD11bGr1 positive cells in the blood and an increase of circulating anti-ENO1 specific antibodies in ENO1+TG100-115 group in comParison to control mice.

Moreover, the analysis of pancreas tissues by immunohistochemistry indicated that combined therapy induced an increased number of CD8 and F4/80 cells and a decrease of FoxP3, CD31 and NG2 cells in comParison to control mice. In addition, we extract mRNA from formalin fixed paraffin embedded pancreas tissues of treated mice. We observed an increase of Granzyme B in both ENO1 and ENO1+TG100-115 and a down modulation of genes involved in fibroblast and stellate cell activation suggesting a modulation of microenvironment in the combined therapy group.

Magnetic Resonance Imaging (MRI) and in particular diffusion-

weighted imaging (DWI) was used to monitor tumor progression. DWI showed that mice vaccinated with ENO1 alone displayed an increase of mean apparent diffusion coefficient (ADC) that significantly decrease after TG100-115 treatment, suggesting an increase in tumor infiltrating cells. ADC is inversely dependent on water movements across the cell membrane and reflects tissue changes at cellular level. Lover ADC values correspond to a higher cellularity that could be due to either tumor progression, immune cell infiltration, increased fibrosis or interstitial edema.

In conclusion, we have demonstrated that treatment with ENO1 plus TG100-115 is able to reduce tumor size in pancreas, increase immune cell infiltration and modulate stroma cell compartment, making the therapy a suitable approach for PDA treatment.

Keywords: Pancreatic cancer, PI3Kgamma inhibitor.

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## B221 / Highly efficient tumor control in a preclinical model for HPV16+ cancer induced by a heterologous prime/boost approach with LCMV- and PICV-based TheraT vectors

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The unprecedented success of checkpoint blockade therapies clearly demonstrated the power of the immune system to fight cancer. Yet, only a minority of cancer patients respond with long-term control of the tumor or even cure, necessitating the development of other treatment modalities. Hookipa Pharma developed a novel attenuated, replication-competent viral vector platform (TheraT) that induces powerful cytotoxic T lymphocyte (CTL) responses against foreign and self-antigens.

In a preclinical model for human papilloma virus 16 (HPV16) associated cancer (TC-1), we evaluated immunogenicity and efficacy of a heterologous prime/boost approach with TheraT vectors based on the arenaviruses lymphocytic choriomeningitis virus (LCMV) and pichinde virus (PICV). Both vectors encode a highly immunogenic, non-oncogenic version of the HPV16 oncoproteins E7 and E6.

Primary E6- and E7-specific CD8 T cell responses were higher after immunization with LCMV-based TheraT compared to PICV-based TheraT. T cells induced by the latter, however, could be boosted 250-fold by a heterologous boost with LCMV-based TheraT. To test, whether the higher T cell response also translates in a better tumor control, mice with established TC-1 tumors were treated with either a homologous or heterologous prime boost regimen. In concordance with the immunogenicity data, tumor control was most efficient in the heterologous setting. Importantly, more than 40% of the animals treated with TheraT(PICV)/TheraT(LCMV) were completely tumor free and were fully protected against a secondary tumor challenge.

In conclusion, replication-attenuated TheraT-E7E6 is safe, highly immunogenic and shows excellent therapeutic efficacy in homologous but even more in a heterologous prime/boost regimen. Preparations for an IND filing in Q1 2020 have been initiated.

Keywords: cancer vaccine, HPV16+ cancer, Arenavirus, heterologous prime/boost.

## B222 / A combinatorial approach of mRNA vaccination and IL-2 with extended half-life enhances antigen-specific T cell responses

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Lipoplex-mediated systemic delivery of RNA encoding cancer-associated antigens to dendritic cells in lymphoid organs is a promising tool for inducing efficient T cell responses in cancer therapy. For further improvement of these anti-tumoral RNA vaccines, combinatorial therapies are under intensive investigation. Immunomodulators, like checkpoint inhibitors and cytokines, are key candidates, since they strongly affect NK and T cell function, and shape tumor microenvironment. Previously, efficient in vivo application of cytokines, as for instance interleukin 2 (IL-2), was characterized by short half-lives and dose-limiting toxicities. In order to attack this problem, we developed modified RNA-encoding murine IL-2 albumin fusion proteins (mAlb-mIL-2) to overcome current limitations and furthermore enhance cytokine potency. In naïve BALB/c and BL/6 mice, we investigated the effect of mAlb-mIL-2 RNA administration on RNA vaccination-induced antigen-specific CD8+ T cell responses.

Application of RNA-encoding mAlb-mIL-2 extended serum half-life of the cytokine and promoted accumulation in the tumor and tumor-draining lymph node in comParison to RNA-derived non-albuminated mIL-2 and recombinant mIL-2 protein. In combination with antigen-encoding RNA vaccination, mAlb-mIL-2 RNA administration was as effective as combinatorial therapy including standard therapy with recombinant mIL-2 concerning the expansion of antigen-specific CD8+ T cells in the blood and spleen, while reducing dose, number of injections as well as risk of toxicity. Notably, combinatorial therapy with mAlb-mIL-2 RNA induced antigen-specific CD8+ T cells, which are more functional, than vaccination alone. Based on these successful proof of principle experiments in naïve mice, the presented combinatorial approach will soon be tested in therapeutic tumor experiments.

In summary, the presented approach of optimized IL-2 is capable of supporting our well-established RNA vaccination platform. Due to its universality, this concept for broadening the therapeutic window of cytokines can be applied to other suitable immunomodulatory components.

Keywords: combination therapy, RNA vaccination, interleukin 2.

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## B223 / Improving selection criteria for post translationally modified CD4 epitopes using computer algorithms

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In tumors, cellular stress and changes in the microenvironment can alter cellular post translational modifications and the epitopes presented by MHC. Targeting cancer associated epitopes produced through stress induced post translational modification (siPTM) is a viable option for cancer immunotherapy. One such siPTM is the conversion of positively changed lysine residues to the neutral amino acid homocitrulline via carbamylation. We have previously shown that peptides containing homocitrulline can induce anti-tumor responses. These peptide responses were HLA-restricted. However, screening of potential epitopes is time consuming with a low hit rate. In this study, computer-based predictions were used to improve the selection of epitopes. Online prediction software was utilized to identify potential HLA-DP4 epitopes based on both MHC class II binding and secondary structures of peptides. The human protein sequences for bind-

ing immunoglobulin protein, alpha-enolase, heat shock protein 60, Nucleophosmin, Cytokeratin 8 and Vimentin were analyzed. IEDB was used to identify peptides with a high predicted binding affinity for the human HLA-DP4 allele. PEP-FOLD3 software was used to analyze peptide predicted secondary structure and narrow down the selection criteria. Selected peptides were then synthesized with either the homocitrulline or wild type lysine residues. Transgenic HLA-HHDII/DP4 mice were immunized with three doses of peptide in combination with the adjuvant CpG/MPLA. IFN$_\gamma$ responses were determined by ex vivo ELISpot. In anti-tumor experiments transgenic mice were implanted with B16 HHDII/DP4 and then immunized with the peptides, tumor growth and survival were monitored. When peptides were selected using IEDB binding alone, 4/10 peptides were found to stimulate an immune response. In contrast selection using the combination of IEDB and Pep-Fold identified 19 with 19/19 giving immune responses. The responses were further characterized to determine cross reactivity with wild type peptides. Of these 6 were screened for anti-tumor responses and all showed anti-tumor immunity in a therapeutic in vivo model. The addition of computer-based algorithms to the peptide selection process greatly increased the number of successful MHC II binding peptides that were identified. This reduced animal usage and increased the number of DP4 presented epitopes that could be characterized.

Keywords: post translational modifications, homocitrulline, binding predictions.

## B224 / Phage-peptide constructs for stimulation of anti-cancer immune responses against CD47

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The long-term goal of this study is to generate effective phage-based products for active immunization against CD47 receptor, a target that is widely used for development of immunotherapies against human and canine cancers. CD47 is a cell surface protein that belongs to the immunoglobulin superfamily and demonstrates high homology in the exposed extracellular-domain between species. It binds several proteins including signal-regulatory protein-alpha (SIRP(alpha)) expressed on phagocytes. Binding of CD47 to SIRP(alpha) leads to inhibition of phagocytosis. In this respect, CD47 plays a role of a “don’t eat me” signal for phagocytic cells, making cells expressing CD47 resistant to phagocytosis. Many types of cancers escape clearance by the immune system by up-regulation of CD47 expression. One strategy to disarm the “don’t eat me” signal on cancer cells is to block CD47 with neutralizing antibodies, preventing the CD47-SIRP(alpha) interaction. The present study is designed to generate and characterize phage-CD47 constructs to stimulate production of blocking antibodies against extracellular CD47 epitopes using phage display technology. Phage-CD47 constructs were obtained via selection from phage display libraries for binding to CD47 antibodies as selection targets. Two types of CD47 antibodies (B6H12 and BRIC126) and three phage display libraries (Ph.D.-C7C, Ph.D.-7, and Ph.D.-12) were used for enrichment of CD47-mimicing peptide sequences. Through this study, we compared any differences in binding epitopes between the two types of CD47 antibodies. We also studied differences in library conformation, comparing the loop-constrained Ph.D.-C7C heptapeptide library with the non-constrained, linear Ph.D.-7 heptapeptide library. After 3-4 rounds of selection with each heptapeptide library, we observed a successive increase in yield from the linear library suggesting population enrichment of phages from the linear library. However, there was no increase observed from the loop-constrained Ph.D.-C7C library suggesting no enrichment and a preference of both antibodies to bind linear peptide epitopes. Similarly, we compared the differences of displayed peptide length on epitope discovery by comparing the Ph.D.-7 library with the Ph.D.-12 library, consisting of linear dodecapeptides displayed on the surface of each phage. We observed an increase in yield from the Ph.D.-12 library suggesting enrichment of phages. After the final round of selection, ~80 clones were randomly isolated for Sanger sequencing and the unamplified portion of each fraction amplified by qPCR with P3-specific primers for analysis of the population by next generation sequencing (NGS). Sequences obtained from Sanger sequencing or NGS were analyzed for their ability to map to the surface of a CD47 crystal structures obtained from the protein database (PDB) using the PepSurf epitope mapping algorithm available on the Pepitope server. Candidate peptides were identified based on their high number of occurrence from the sequenced clones or were found at the proposed interaction site between CD47 and SIRP(alpha). In total, 12 peptides were identified and synthesized as peptides. Peptides were adsorbed to an ELISA plate and tested for their performance to bind either CD47 antibody in an indirect ELISA assay. We discovered 3 peptides with relatively high binding activity to both CD47 antibodies tested suggesting the antibodies may share a common structural epitope. The outcomes of the proposed study should provide a platform for continued characterization of phage-CD47 constructs for anti-cancer efficacy in various models of human cancers and in dogs with cancers such as spontaneous lymphomas. This research may lead to the development of novel, active anti-cancer immunotherapies applicable to both human and veterinary patients.

Keywords: Phage Display, CD47 Vaccine, Active Immunization, Cancer Vaccination.

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## B225 / In-situ vaccination using a polymeric glyco-adjuvant for the induction of anti-tumor immunity

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Development of new immunotherapies capable of effecting durable anti-tumor responses is a long-term goal in the field of cancer immunotherapy. Neoantigen-peptide based vaccines have demonstrated efficacy in murine tumor models, however, clinical translation is limited by the costly and slow process of identifying tumor and/or patient-specific immunogenic antigens prior to formulation. Difficultly translating therapeutic vaccination success from murine models to the clinical treatment of cancer has highlighted the need for vaccination strategies that may be more broadly applicable. To address this problem, we developed an in situ vaccination strategy to adjuvant tumor cells directly, utilizing the tumor itself as the antigen source in initiating tumor-reactive cellular responses. Our engineered vaccine is comprised of a novel polymeric TLR7-agonist (pTLR7) chemically linked to tumor-binding antibodies. We hypothesized that following intratumoral administration, our pTLR7-(tumor-binding antibody) conjugates (pTLR7-tAbs) would bind to surface molecules expressed by tumors, thereby increasing persistence of the adjuvant within the tumor. Given the ease of conjugation of pTLR7 to various antibodies, our in situ pTLR7-tAb vaccination platform is tunable in specificity from highly tumor-specific to widely applicable, depending on the choice of antibody used. For generalizability between tumor models, we selected an anti-CD47 antibody to create anti-CD47-pTLR7 conjugates for testing our vaccination. Consistent with previous reports of elevated CD47 expression in various malignancies across both human and mouse, flow cytometric analysis confirmed the ability of anti-CD47-pTLR7 to bind to tumor cells from every tumor model we tested: B16F10 melanoma, EMT6 and PyMT breast cancer, and genetically engineered melanoma model BrafV600E/Ptenfl/fl. Tumor retention studies tracking fluorescently-labeled pTLR7-tAbs using an in vivo imaging system (IVIS) showed pTLR7-tAbs are retained within the tumor microenvironment (TME) 3-fold longer than isotype control antibody-pTLR7 constructs. Interestingly, following vaccination, pTLR7-tAb treated tumors showed increased accumulation and

activation of intratumoral antigen presenting cells (APCs). pTLR7-tAb vaccination stimulated tumor-specific T cell responses in the B16F10 melanoma model and converted these immunologically 'cold' tumors into T cell inflamed tumors, with increased numbers of CD4+ and CD8+ T cells. Strikingly, we observed complete remission in >50% of 'cold' immune-excluded EMT6 breast cancer tumors and delayed tumor progression of B16F10 melanoma after treatment with pTLR7-tAbs. As intratumorally retained pTLR7-tAbs showed improved tumor control over equimolar, unconjugated mixtures of antibody and pTLR7, antibody-linkage and tumor retention of pTLR7 appeared important for therapeutic efficacy. Together, these data support further investigation into the use of our pTLR7-tAb platform for in situ vaccination and as a therapeutic tool for enhancing anticancer immunity.

Keywords: cancer vaccine, vaccine, adjuvant.

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Wilson, D. S., Hirosue, S., Raczy, M. M., Bonilla-Ramirez, L., Jeanbart, L., Wang, R., et al. (2019). Antigens reversibly conjugated to a polymeric glyco-adjuvant induce protective humoral and cellular immunity. Nature Materials, 18(2), 175–185.

## B226 / HEPAVAC-101 first-in-man clinical trial of a multi-peptide-based vaccine for hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the third leading cause of death from cancer globally with an extremely variable 5-year survival rate. Immunotherapy strategies for HCC may represent a key therapeutic tool to improve clinical outcome in HCC patients. HepaVac-101 (EudraCT Number: 2015-003389-10; NCT03203005), is a single-arm, first-in-man Phase I/II clinical trial evaluating a therapeutic cancer vaccine in patients affected by HCC. It is a highly innovative, novel approach based on a multi-peptide vaccine (IMA970A) combined with an RNAdjuvant (CV8102). The IMA970A off-the-shelf vaccine includes 5 HLA-A*24 and 7 HLA-A*02 as well as 4 HLA-DR restricted peptides identified and selected from native human HCC tumor tissue by applying the XPRESIDENT® discovery platform. CV8102 is a novel ribonucleic acid (RNA) based immunostimulatory agent inducing a balanced Th1/Th2 immune response. HLA-A*02 and/or A*24-positive patients with very early, early and intermediate stage HCCs are being enrolled to be treated with 9 intradermal vaccinations consisting of IMA970A plus CV8102 following a single pre-vaccination infusion of low-dose cyclophosphamide acting as an immunomodulator. The first 4 vaccinations are applied in weekly intervals, while the following 5 vaccinations are administered in 3-weekly intervals. Study drugs are applied without concomitant anti-tumor therapy aiming to reduce the risk of tumor recurrence/progression in patients who have received all indicated treatments according to the standard of care and remain without evidence of active disease that warrants further treatment. The primary endpoints of the HepaVac-101 clinical trial are safety, tolerability, and immunogenicity. Secondary/exploratory endpoints are additional immunological parameters in blood, infiltrating T-lymphocytes in tumor tissue, biomarkers in blood and tissue, disease-free survival/progression-free survival and overall survival. Patients were enrolled in 6 centers located in 5 European countries i.e. Italy (Naples and Negrar/Varese), Germany (Tübingen), UK (Birmingham), Spain (Pamplona) and Belgium (Antwerp). At the time of abstract submission, 82 HCC patients have been screened for suitable HLA haplotypes, 21 patients were put on study treatment (i.e. received at least the pre-treatment with cyclophosphamide) and 10 out of them have already completed study treatment and are currently in the follow-up phase. The clinical study protocol stated that 20 patients treated with IMA970A and CV8102 are considered sufficient to evaluate the trial endpoints of this trial and for that reason screening of new patients was officially closed. Eligible patients will be eventually invited to participate in a successive trial at Tübingen University investigating an actively personalized vaccine (APVAC). So far, the observed safety profile is as expected.

The HEPAVAC-101 clinical trial is the achievement of the HEPAVAC Consortium supported by the European Commission's 7th framework program with contract No. 602893 (www.hepavac.eu).

Keywords: Liver Cancer, Cancer Vaccine.

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Buonaguro L; HEPAVAC Consortium. New vaccination strategies in liver cancer. Cytokine Growth Factor Rev. 2017 Aug;36:125-129. doi: 10.1016/j.cytogfr.2017.06.010. Epub 2017 Jun 23. Erratum in: Cytokine Growth Factor Rev. 2017 Sep 12;:. PubMed PMID: 28688773.

## B227 / Personalized cancer vaccination to elicit neoantigen T cell responses.

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Despite great improvements being achieved in cancer diagnosis and treatments, a significant fraction of patients remains unresponsive to standard therapies and still have short life expectancies. With more than a hundred distinct types of cancer known that vary substantially in behaviors, cancer treatment is increasingly geared towards a personalized approach. In particular, immunotherapy aiming at eliciting and/or boosting patient's anti-tumor immune responses is an attractive approach. An important question in the field of tumor immunotherapy remains unanswered – what are the relevant tumor antigens for tumor control and elimination? To date, the most widely used targets are tumor-associated antigens (TAAs). These TAAs vary in terms of expression levels, tissue specificity and their ability to induce tumor-infiltrating lymphocytes (TILs) in the tumor microenvironment. However, they pose some limitations such as central tolerance via thymic selection and tumor escape through antigen loss. On the other hand, neoantigens are mutated antigens that are specific to the tumor and hence are interesting targets for anti-cancer immunotherapy strategies like cancer vaccination. Numerous preclinical and clinical studies have demonstrated the correlation between tumor high mutational burden and clinical benefits in terms of long-term survival. Currently, neoantigens prediction is based on expensive high-throughput technologies like next generation sequencing, mass spectrometry and sophisticated neoepitope discovery algorithms. These procedures are time-consuming and laborious rendering this strategy unsuitable for cancer patients with advanced disease. An alternative strategy is the use of hypochlorous acid (HOCl)-oxidized whole tumor lysate (WTL) that encompasses all the tumor antigens including shared TAAs and private mutated neoantigens. Treating tumor cells with HOCl oxidation has multiple advantages such as potentiating their immunogenicity via chemical modifications, leading to the exposure of immunogenic peptides to T lymphocytes and their subsequent activation. Recently, our group demonstrated in a clinical study with recurrent ovarian cancer patients that dendritic cell (DC) pulsed with WTL (termed OCDC) could elicit high-avidity neoantigen-specific T cell responses and these results were associated with prolonged survival. We also demonstrated that upfront vaccination with OCDC elicited de-novo T cell responses against previously unrecognized neoantigens. Based on these results we propose to use OCDC vaccination as a first-line strategy to elicit neoantigen-specific T cells that will lead to a more precise identification of relevant immunogenic neoepitope for anti-cancer neoantigen-based vaccination . In this study, we used three

distinct murine cancer types: the Lewis lung cancer (LLC1), melanoma (B16F10) both known to have a high mutational load and the ovarian cancer model (ID8) known to have a low mutational burden. We successfully showed that our antigen identification algorithms were able to identify immunogenic neoantigens that elicit tumor suppression in LLC1 and B16.F10 tumor models. Most of the neoantigens discovered with our in-house neoantigen discovery pipeline were not yet described, therefore highlighting the novelty of this work. Secondly, we discovered overlapping neoantigen peptide responses between OCDC vaccination and neoantigen peptide vaccination in the LLC1 model. Finally, in the LLC1 tumor models whereby OCDC vaccination gave the best overall tumor suppression, we discovered common neoantigen peptide responses between OCDC and peptide vaccinations as well as unique neoantigen responses induced only by OCDC vaccination. Such a strategy is novel and is potentially applicable to many patients regardless of HLA haplotypes and in different cancer types including those with intermediate/ low mutational burden.

Keywords: Tumor neoantigens, Autologous whole tumor lysate, Dendritic cell vaccine.

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## B228 / Development of technology for the large scale production of a novel dendritic cell vaccine for the treatment of glioblastoma

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Glioblastoma multiform (GBM), is one of the most lethal and untreatable human cancers (1-5). Surgical resection and radiotherapy in combination with temozolomide (TMZ) are the current therapeutic approaches, but they offer little hope with five year survival rates lower than 5%. With this poor prognosis, it is clear that new strategies are needed for glioblastoma therapy (5-8).

The Mayo Clinic in Rochester, MN has developed an autologous Dendritic Cell Vaccine (DCV), which was evaluated in a phase I clinical trial for newly diagnosed GBM patients. The vaccine technology is comprised of two components: Allogeneic tumor lysates with classic GBM antigens and autologous dendritic cells capable of inducing an immune response. Critically, the combined allogenous-lysate/autologous-DC approach allows the limitations of other DC vaccine approaches to be overcome, such as the delays required for autologous tumor cell culturing. The technology offers a maximum number of fully mature DCs, as well as a full repertoire of GBM antigens, allowing for effective targeting and destruction of GBM cells.

This therapy was recently used in combination with radiation and temozolomide in a phase I clinical trial (MC1272) with 20 patients. Median progression-free (PFS) and overall survivals (OS) were significantly prolonged vs historical controls treated with radiation and temozolomide alone.

Mill Creek Life Sciences, LLC (MCLS) has the license to commercialize this technology. The cell based cancer immunotherapy/ vaccine requires processes to prepare antigenic proteins from tumor cell lines, to obtain patient's dendritic cells and prime them with the antigenic proteins, and to deliver the primed dendritic cells to patients being treated for glioblastoma.

Our company is scaling the antigen protein preparation from 2D culture of GBM cells to suspension culture in bioreactors. The use of allogeneic tumor cell lines, avoids delays in treatment caused by the time required to culture an autologous tumor cell line. Classical cell culturing Methods are ineffective for GBM propagation. Our unique and proprietary method for generating GBM cell lines more closely mimics the native microenvironment of GBM, reduces senescence, and maintains a robust and defined tumor antigen expression.

The dendritic cell preparation is also unique. Standard culture techniques produce > 90% mature (CD83+) DCs from healthy donor monocytes, but < 60% mature DCs from GBM patients' monocytes. However, the modified technique developed at the Mayo Clinic produces > 90% mature DCs from GBM patients.

After successfully transferring the technology from Mayo Clinic and adapting it for large scale production, our long-term goal is to effectively produce and commercialize the therapy for the treatment of patients with glioblastoma in a clinical environment.

Commercial production of our vaccine therapy will pave the way toward future GBM clinical trials. This treatment has the potential to revolutionize GBM management and may become a part of standard treatment regimens for GBM patients. Given successful implementation in further clinical trials, we will proceed to develop DC vaccines for a variety of other severe forms of cancer. The future of anticancer immunotherapy is very bright, and innovation in DC vaccine technology will provide avenues toward better management of GBM, as well as many other cancers and malignancies.

Keywords: cancer vaccine, Glioblastoma multiform, dendritic cells.

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## B229 / Personalized neoantigen-targeting vaccines for high-risk melanoma generate long-term memory T cell response and epitope spreading

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Cancer vaccines have been envisioned as an effective tool to generate, amplify, and diversify T cell responses against tumors. Tumor neoantigens are key targets of effective anti-tumor immune responses. Recently, we have demonstrated that a neoantigen vaccine (NeoVax), consisting of up to 20 long peptides and poly-ICLC, induced strong polyfunctional neoantigen-specific T cells that recognized patient tumor in vitro.

All 6 patients we initially reported on (Ott &Wu, Nature, 2017) and 2 new patients are alive. Complete responses in the 2 patients with stage IV melanoma who received pembrolizumab are ongoing. Three of 4 patients with stage III melanoma had tumor recurrences; 2 of these 3 patients underwent re-resection and are with-

out evidence of disease (NED), while one patient is on treatment for unresectable metastatic disease. An additional 2 patients with stage III melanoma received NeoVax since the initial report and are NED after 2 and 3 years, respectively. To evaluate the long-term effects of the vaccine, we have analyzed the persistence of neoantigen-specific T cell responses at 3-4 years post-vaccination for 4 patients. Between 17-100% of CD4+ and 50-100% of CD8+ T cell responses against neoantigen peptides detected at week 16 persisted at 3-4 years post-vaccination. We also confirmed that the vaccine induced neoantigen-specific CD4+ and CD8+ T cell responses in the 2 new patients. Hypothesizing that vaccine-induced tumor destruction leads to release of additional neoantigens that can trigger further tumor-specific immune responses, we tested for T cell reactivity against neoepitopes that were ranked high by the epitope selection pipeline and were not included in the personalized vaccines as well as tumor associated antigens (TAAs) that were also not included in the vaccines in 3 patients. For the assessment of CD8+ T cell responses, we designed peptides (predicted by NetMHCpan) arising from 2 categories of antigens: (i) neoantigen peptides; (ii) TAA peptides based on high tumor gene expression. For testing of CD4+ T cell responses, we designed peptides that spanned predicted neoepitopes from category i. We tested CD8+ T cells against 123 neoantigens and 87 TAAs, and CD4+ T cells against 30 neoantigens from all 3 patients. We identified de novo CD4+ T cells responses against 3 peptides (mut-AGAP3, -EYA3, -P2RY4) not included in the original respective vaccines at week 16. An additional CD4+ T cell response against a different neoantigen peptide derived from mut-AGAP3 was detected only after CPB. We also identified CD8+ T cells specific for MAGE F and MAGE D in the week 16 and after CPB therapy samples, respectively. Lastly, all four lines of CD4+ T cells reactive against these identified neoantigens were able to discriminate between the mutated and wildtype forms of the peptides. We also performed longitudinal assessment of T cell receptor (TCR) clonotypes as well as whole transcriptome sequencing on neoantigen tetramer sorted single CD4+ T cells. In 2 patients, the top 3 dominant clonotypes persisted between week 3-24 post-vaccination; furthermore, we observed expansion and diversification of clonotypes over time. In one patient tested to date for single cell trancriptomics, neoantigen-specific T cells expressed multiple activation and memory genes between week 3-24. Exhaustion genes were expressed only after week 16 post-vaccination. Taken together, these data demonstrate that T cell responses induced with personalized neoantigen peptide vaccines in melanoma patients are durable over several years, expand and diversify over time as assessed on a single cell clonal level, and may trigger tumor cell killing as indicated by epitope spreading.

Keywords: Neoantigen, TCR sequencing, epitope spreading, memory T cells.

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An immunogenic personal neoantigen vaccine for patients with melanoma, Nature, 2017

## B230 / Targeting a polymeric glyco-adjuvant to the tumor microenvironment via collagen affinity slows tumor progression

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Cancer vaccines are one form of immunotherapy that utilize tumor proteins, or antigens, to activate cellular and humoral immune responses against the tumor. Typically, these vaccines are comprised of specific antigens along with immunostimulatory adjuvants. However, successful cancer vaccination is challenging, as there is a lack of strong, clinically approved adjuvants and it is difficult to identify cancer-specific antigens. Here, in order to address these challenges, we report the creation of a targeted therapeutic cancer vaccine using a mannosylated, toll-like receptor 7 (TLR7)-agonizing polymer (p(Man-TLR7)) conjugated to a fusion protein consisting of serum albumin (SA) fused to a collagen binding domain (CBD), namely the A3 domain of von Willebrand factor. We have previously shown that our polymeric glyco-adjuvant is capable of eliciting strong CD8+ T cell responses in traditional model antigen or malaria vaccination models due to its design. The mannose component enhances uptake by various mannose-recognizing C-type lectin receptors on antigen presenting cells (APCs). This routes antigen for cross-presentation to CD8+ T cells and also shuttles the adjuvant polymer to the endosome, where it can activate TLR7. TLR7 signaling is important for the production of cytokines and other soluble immune activating molecules that promote cross-presentation to and priming of CD8+ T cell responses. As CD8+ T cell responses are critical for the therapeutic efficacy of cancer vaccines, we hypothesized that p(Man-TLR7) would provide the appropriate APC activation and cytokine environment for use in a cancer vaccine. CBD-SA combines the active targeting of the CBD protein to the tumor via binding to collagen in the tumor microenvironment, as we have previously reported, with the known passive targeting of SA. Thus, by chemically linking the p(Man-TLR7) glyco-polymer to CBD-SA, our engineered material aims to localize this strong adjuvant to the tumor microenvironment upon intravenous injection, promoting the immunogenic processing of endogenous tumor antigens, bypassing the need to pre-identify tumor-specific antigens. Using our previously published conjugation strategy, we were able to chemically link p(Man-TLR7) to free amines on CBD-SA, as verified by observed shifts in protein molecular weight at each step of conjugation. Production of CBD-SA-p(ManTLR7) conjugates has been reproducible and allows for the consistent generation of vaccine materials with little variability. Importantly, when CBD-SA was conjugated to p(Man-TLR7), the CBD-SA-p(Man-TLR7) conjugates retained the ability to bind to both collagen I and collagen III. We then assessed the anti-tumor efficacy in vivo, both as a monotherapy and in combination with checkpoint antibody therapy. We hypothesized that our vaccination would achieve maximal therapeutic efficacy with checkpoint blockade therapy, as this would allow our vaccine to overcome T regulatory cell or PD-L1 mediated immunosuppression. As such, we explored synergy with a combination of anti-PD-1 and anti-CTLA4 antibodies in particular. Treatment of B16F10 tumor-bearing mice with intravenously-delivered CBD-SA-p(Man-TLR7) alone resulted in a 60% decrease in average tumor size compared to untreated controls. Further improved anti-tumor efficacy (an 85% decrease in average tumor size compared to untreated controls) and improved overall survival were observed when we combined our CBD-SA-p(Man-TLR7) vaccination with anti-PD-1 and anti-CTLA-4 antibodies.

In conclusion, this data shows that CBD-SA-p(Man-TLR7) vaccination in combination with checkpoint antibody therapy provides therapeutic benefit in this treatment of a poorly immunogenic melanoma model.

Keywords: vaccine adjuvant, targeted immunotherapy, TLR7 agonist.

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Wilson, et al. Nat Mater 2019;18:175-185. Ishihara, et al. Sci Transl Med 2019;11:eaau3259.

## B231 / Reprogrammed tumor microenvironment by intratumoral LCMV vector therapy promotes T cell-dependent melanoma eradication

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Tumor cells grow in a complex and heterogeneous tumor microenvironment (TME) that consists of diverse cellular components including immune cells, stromal fibroblasts and endothelial cells. The TME can influence antitumor immune responses and tumor outcome. Therefore, targeting the TME has become one of the main goals in anti-cancer therapy. Here, we demonstrate that a single intravenous or intratumoral administration of a recombinant replication –attenuated LCMV vector expressing the melanoma-associated antigen TRP2 (TheraT(LCMV)-Trp2) confer T-cell dependent control of melanomas, whereas only intratumoral but not intravenous injection of the vector leads to T cell-dependent eradication of melanoma. Despite the high number of tumor infiltrating T cells in intravenously treated mice, this did not correlate with tumor control. However, we found preservation of T cell functionality in mice treated intratumorally with TheraT(LCMV)

vectors. This suggests that intratumoral injection of TheraT(LCMV)-Trp2 vectors reprogram the TME to sustain T cell fitness. Using single cell transcriptomics, we are currently setting out to dissect the changes in the TME after intratumoral TheraT(LCMV) treatment. Thereby, we are going to analyze cell populations in the hematopoietic as well as the non-hematopoietic compartment. Collectively, our data show that intratumoral injection of TheraT(LCMV) vectors is an efficient way of reprograming the TME landscape to enhance antitumor T cell responses resulting in tumor eradication.

Keywords: Virotherapy.

## B232 / Short linear and microbe-derived Peptides enhance T-cells and anti-tumor activity

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Monoclonal antibody inhibitors of checkpoint receptors, particularly PD1 and CTLA4, have proved to be powerful tools for treating cancer. Additionally, the combination of these checkpoint inhibitors could be advantageous and many such combination trials of monoclonal antibodies are underway. However, inhibition of PD1 and CTLA4 through therapeutic antibodies also comes with a cost of increased safety issues related primarily to autoimmune adverse reactions. Moreover, combination therapies will add to the cost of these already expensive modalities. We have created a platform, MicrotideTM, for discovering short peptides that bind the checkpoint receptors by screening peptide libraries and rapidly evolving the peptide scaffolds by in silico design. Here, we report the discovery, invitro characterization, and invivo proof of concept of our lead peptide-based checkpoint immunomodulators (LD-series). These peptides appear to have the polypharmacological properties of simultaneously antagonizing at least two of the CD28 family of receptors, namely PD1 and CTLA4. We demonstrated that the LD peptides induce T cell proliferation, enhance T cell activity in PBMCs, have potent antitumor activity in mouse models, and promote vaccine-induced T cells with as little as a single LD peptide administration. In addition, we have generated RNA, DNA, and virus constructs encoding the LD peptides to demonstrate the feasibility and advantage of our peptides to be effectively delivered locally in the tumor microenvironment. Our peptides have shown great potential as a novel, polypharmacological immunotherapies that can be cost effectively incorporated into, and combined with, other modalities such as cellular therapies, oncolytic viruses, and cancer vaccines to safely deliver anti-tumor efficacy.

Keywords: checkpoint immune modulator, t-cell proliferation, cancer vaccine combination, peptide.

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## B233 / Relevance and modulation of RNA-triggered vaccine adjuvanticity for T cell immunity

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Vaccination with liposomal antigen-coding messenger RNA has proven a promising tool to induce tumor-specific T cell immunity. Interestingly, RNA vaccination triggers an inflammatory environment characterized by strong IFNα network activation demonstrated to be required for the induction of functional anti-tumoral T cells. As triggering innate immune mechanisms is crucial for the induction of a potent T cell response, we characterized vaccine RNA-mediated adjuvanticity in more detail by varying the antigen RNA dose and decoupling the dual role of RNA as carrier of antigen information and as provider of adjuvant activity.

We showed that the antigen RNA dose correlated with the expression of stimulation- and migration-associated molecules on DCs as well as activation markers on T cells in the spleen. In addition, RNA vaccination triggered the release of pro-inflammatory cytokines and chemokines in a dose-dependent manner, indicating extensive immune activation not only restricted to cells directly in contact with RNA. In agreement with the DC and T cell activation status, the frequency of antigen-specific CD8+ T cells was equally antigen RNA dose-dependent. Interestingly, T cell differentiation, indicated by KLRG1 upregulation on antigen-specific CD8+ T cells, correlated positively with antigen RNA dose, with higher doses promoting T cell differentiation towards effector T cells. We dissected the roles of RNA-mediated adjuvant activity and antigen availability further by vaccinating mice with increasing doses of antigen RNA combined with irrelevant RNA to a fixed total RNA dose. As expected, the extent of splenic immune cell activation was determined solely by the total RNA dose. Interestingly, mainly the adjuvant effect rather than the antigen dose determined the increase in frequency of CD8+ T cells and promoted their differentiation into effector cells, In line with the importance of the adjuvant role, addition of escalating doses of irrelevant RNA to a fixed antigen RNA dose boosted the overall strength of T cell immunity.

In summary, our data demonstrate that RNA-encoded antigen information can be separated from RNA intrinsic adjuvanticity, and that the adjuvant activity of RNA vaccines influences the breadth and the phenotype of the induced T cell immunity. As a direct result, antigen RNA dose can be kept to a minimum and adjuvanticity can be modulated according to need by the addition of irrelevant RNA.

Keywords: vaccination, vaccine adjuvanticity.

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