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ITOC7 - THE 7TH LEADING INTERNATIONAL CANCER IMMUNOTHERAPY CONFERENCE IN EUROPE

2–3 October 2020 - VIRTUAL CONFERENCE

ABSTRACTS

THE LEADING INTERNATIONAL CANCER IMMUNOTHERAPY CONFERENCE IN EUROPE

7th ImmunoTherapy of Cancer Conference
2–3 October 2020, VIRTUAL CONFERENCE

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Live Talks

L1 TGF-BETA BLOCKS TYPE I IFN RELEASE AND TUMOR REJECTION IN SPONTANEOUS MAMMARY TUMORS

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Background Activation of the STimulator of INterferon Genes (STING) by DMXAA (5,6-dimethylxanthenone-4-acetic acid) can induce a strong production of IFNα/β and the rejection of transplanted primary tumors. However, the efficacy of such therapeutic approach for the treatment of spontaneous tumors had still to be evaluated.

Material and Methods We have tested whether the injection of DMXAA or other STING agonists and TLR4 agonist, could lead to the regression of spontaneous MMTV-PyMT mammary tumors. We also characterized, in time and space, the early signaling events triggered downstream STING and the distribution of infiltrating immune cells in the tumor microenvironment by fluorescence imaging.

Results We show that spontaneous MMTV-PyMT mammary tumors are resistant to immunotherapeutic intervention. We demonstrate that TGFβ, abundant in spontaneous tumors, is a key molecule limiting this IFN-induced-tumor regression by DMXAA. Mechanistically, we found that TGFβ blocks the phosphorylation of IRF3 and the ensuing IFNα/β production by tumor infiltrating macrophages. Finally, blocking TGFβ restores the production of IFNα by activated $MHCII^+$ tumor-associated macrophages, and enables tumor regression induced by STING activation.

Conclusions Based on these findings, we propose that the efficacy of many cancer therapies, which are type I IFN-dependent, should be greatly improved by combination with TGFβ blockade.

Disclosure Information N. Bercovici: None. **M.V. Guérin:** None. **F. Regnier:** None. **J.M. Weiss:** None. **V. Feuillet:** None. **L. Vimeux:** None. **G. Altan-Bonnet:** None. **E. Donnadieu:** None. **A. Trautmann:** None.

L2 IN VIVO LIVE IMAGING OF HUMAN T/B CELL LYMPHOMA CROSS-LINKING MEDIATED BY BISPECIFIC CD20-TCB ANTIBODY

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10.1136/jitc-2020-ITOC7.2

Introduction Cancer Immune Therapies have shown unprecedented results in improving tumor control.[1–3] However, many patients are still refractory to treatment. A deeper understanding of the mode of action of the different CITs sub-classes may help improving therapeutic approaches to reach better anti-tumor response. For this reason, we developed a multi-photon intra-vital microscopy (MP-IVM) approach to study *in vivo*, at single cell level, the tumor microenvironment upon treatment with CD20-targeting T-cell bispecific antibodies (TCB) [4] in a preclinical model of diffuse large B cell lymphomas (DLBCL).

Methods To selectively monitor clinical lead molecules in the context of human T cell responses, we developed a skinfold chamber model [5] in last generation humanized mice [6] that allows visualization, by MP-IVM, of labelled human T cells co-injected intra-dermally with WSU-DLCL2, a human DLBCL. We have used this model to investigate T cells recruitment to tumors upon CD20-TCB therapy: by intravenously injecting labeled T cells in mice treated with selected blocking antibodies, we were able to identify dedicated pathways induced by CD20-TCB and regulating T cell influx into the tumor bed. Furthermore, we developed a user-independent quantification platform to assess changes in the dynamics of T cell motility and time of interaction with tumor cells.

Results/Discussion We have developed an experimental preclinical model that aims to reduce xenoreaction (human T cell reaction against mouse tissue) by utilizing T cells derived from humanized mice, educated within murine thymus. We demonstrate that such model is optimal to quantify human T cell dynamics *in vivo*. We show that CD20-TCB localizes in the tumor and acts on tumor-resident T cell motility within 1 hour post i.v. injection (defined as functional PK), causing a sharp reduction in their speed (from 4 to 2 μm/min) and an increase in tumor/T cell interaction time; those changes last up to 72h post-treatment. In addition, we prove how the initial tumor/T cell interaction mediated by CD20-TCB lead to peripheral T cells recruitment into the tumor. This mechanism is dependent on the presence of tumor-resident T cells and on IFNg-CXCL10 pathway. Inhibiting any of these two parameters resulted in reduced T cells infiltration from the periphery and reduced anti-tumor efficacy.

Conclusion We developed a reliable imaging and analysis pipeline to investigate *in vivo* T cell dynamics and recruitment and applied it to the study of CD20-TCB treatment of DLBCL model. Our approach has shed new lights into the MoA of this new class of immune-therapeutics, demonstrating that the IFN□-CXCL10 pathway is involved in T cell recruitment upon CD20-TCB treatment.

Disclosure Information F. Cremasco: A. Employment (full or part-time); Significant; Roche Glycart AG. **E. Menietti:** A. Employment (full or part-time); Significant; Roche Glycart AG. **D. Speziale:** A. Employment (full or part-time); Significant; Roche Glycart AG. **J. Sam:** A. Employment (full or part-time); Significant; Roche Glycart AG. **S. Sammicheli:** A. Employment (full or part-time); Significant; Roche Glycart AG. **S. Chen:** A. Employment (full or part-time); Significant; Roche Glycart AG. **M. Richard:** A. Employment (full or part-time); Significant; Roche Glycart AG. **C. Klein:** A. Employment (full or part-time); Significant; Roche Glycart AG. **P. Umana:** A. Employment (full or part-time); Significant; Roche Glycart AG. **M. Bacac:** A. Employment (full or part-time); Significant; Roche Glycart AG. **S. Colombetti:** A. Employment (full or part-time); Significant; Roche Glycart AG. **M. Perro:** A. Employment (full or part-time); Significant; Roche Glycart AG.

L3 UPDATE OF THE OPACIN AND OPACIN-NEO TRIALS: 36-MONTHS AND 24-MONTHS RELAPSE-FREE SURVIVAL AFTER (NEO)ADJUVANT IPILIMUMAB PLUS NIVOLUMAB IN MACROSCOPIC STAGE III MELANOMA PATIENTS

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10.1136/jitc-2020-ITOC7.3

Background Before adjuvant checkpoint inhibition the 5-year overall survival (OS) rate was poor (<50%) in high-risk stage III melanoma patients. Adjuvant CTLA-4 (ipilimumab, IPI) and PD-1 (nivolumab, NIVO, or pembrolizumab) blockade have been shown to improve relapse-free survival (RFS) and OS (latter only for IPI so far). Due to a broader immune activation neoadjuvant therapy with checkpoint inhibitors might be more effective than adjuvant, as suggested in preclinical experiments. The OpACIN trial compared neoadjuvant versus adjuvant IPI plus NIVO, while the subsequent OpACIN-neo trial tested three different dosing schedules of neoadjuvant IPI plus NIVO without adjuvant therapy. High pathologic response rates of 74–78% were induced by neoadjuvant IPI plus NIVO. Here, we present the 36- and 24-months RFS of the OpACIN and OpACIN-neo trial, respectively.

Materials and Methods The phase 1b OpACIN trial included 20 stage IIIB/IIIC melanoma patients, which were randomized to receive IPI 3 mg/kg plus NIVO 1 mg/kg either adjuvant 4 cycles or split 2 cycles neoadjuvant and 2 adjuvant. In the phase 2 OpACIN-neo trial, 86 patients were randomized to 2 cycles neoadjuvant treatment, either in arm A: 2x IPI 3 mg/kg plus NIVO 1 mg/kg q3w (n=30), arm B: 2x IPI 1 mg/kg plus NIVO 3 mg/kg q3w (n=30), or arm C: 2x IPI 3 mg/kg q3w followed immediately by 2x NIVO 3 mg/kg q3w (n=26). Pathologic response was defined as <50% viable tumor cells and in both trials centrally reviewed by a blinded pathologist. RFS rates were estimated using the Kaplan-Meier method.

Results Only 1 of 71 (1.4%) patients with a pathologic response on neoadjuvant therapy had relapsed, versus 16 of 23 patients (69.6%) without a pathologic response, after a median follow-up of 36 months for the OpACIN and 24 months for the OpACIN-neo trial. In the OpACIN trial, the estimated 3-year RFS rate for the neoadjuvant arm was 80% (95% CI: 59%-100%) versus 60% (95% CI: 36%-100%) for the adjuvant arm. Median RFS was not reached for any of the arms within the OpACIN-neo trial. Estimated 24-months RFS rate was 84% for all patients (95% CI: 76%-92%); 90% for arm A (95% CI: 80%-100%), 78% for arm B (95% CI: 63%-96%) and 83% for arm C (95% CI: 70%-100%). Baseline interferon-γ gene expression score and tumor mutational burden predict response.

Conclusions OpACIN for the first time showed a potential benefit of neoadjuvant IPI plus NIVO versus adjuvant immunotherapy, whereas the OpACIN-neo trial confirmed the high pathologic response rates that can be achieved by neoadjuvant IPI plus NIVO. Both trials show that pathologic response can function as a surrogate markers for RFS.

Clinical trial information NCT02437279, NCT02977052

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L4 SYNTHETIC AGONISTIC RECEPTOR-ACTIVATING BITES – A MODULAR PLATFORM FOR THE EFFICIENT TARGETING OF ACUTE MYELOID LEUKEMIA

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10.1136/jitc-2020-ITOC7.4

Background Targeted immunotherapies have shown limited success in the context of acute myeloid leukemia (AML). Due to the mutational landscape and heterogeneity attributed to this malignancy and toxicities associated with the targeting of myeloid lineage antigens, it has become apparent that a modular and controllable cell therapy approach with the potential

to target multiple antigens is required. We propose a controlled ACT approach, where T cells are armed with synthetic agonistic receptors (SARs) that are conditionally activated only in the presence of a target AML-associated antigen, and a cross-linking bispecific T cell engager (BiTE) specific for both (SAR) T cell and tumour cell.

Materials and Methods A SAR composed of an extracellular EGFRvIII, trans-membrane CD28, and intracellular CD28 and CD3z domains was fused via overlap-extension PCR cloning. T cells were retrovirally transduced to stably express our SAR construct. SAR-specific bispecific T cell engagers (BiTE) that target AML-associated antigens were designed and expressed in Expi293F^{TM}cells and purified by nickel affinity and size exclusion chromatography (SEC). We validated our approach in three human cancer models and patient-derived AML blasts expressing our AML-associated target antigen CD33.

Results CD33-EGFRvIII BiTE, monovalently selective for our SAR, induced conditional antigen-dependent activation, proliferation and differentiation of SAR-T cells. Further, SAR T cells bridged to their target cells by BiTE could form functional immunological synapses, resulting in efficient tumor cell lysis with specificity towards CD33-expressing AML cells. SAR.BiTE combination could also mediate specific cytotoxicity against patient-derived AML blasts whilst driving SAR T cell activation. *In vivo*, treatment with SAR.BiTE combination could efficiently eradicate leukemia and enhance survival in an AML xenograft model. Furthermore, we could show selective activation of SAR T cells, as well as a controllable reversibility of said activation upon depletion of the T cell engaging molecule.

Conclusions Here we apply the SAR x BiAb approach in efforts to deliver specific and conditional activation of agonistic receptor-transduced T cells, and targeted tumour cell lysis. The modularity of our platform will allow for a multi-targeting ACT approach with the potential to translate the ACT successes of B cell malignancies to AML. With a lack of truly specific AML antigens, it is invaluable that this approach possesses an intrinsic safety switch via its BiTE facet. Moreover, we are able to circumvent pan-T cell activation due to the specific targeting and activation of SAR T cells.

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L5 RIG-I ACTIVATION ENHANCES MELANOMA IMMUNOGENICITY AND IMPROVES ANTI-TUMOR T CELL RESPONSES IN COMBINATION WITH ANTI-PD-1 IMMUNE CHECKPOINT BLOCKING ANTIBODIES

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10.1136/jitc-2020-ITOC7.5

Background Clinical efficacy of immune checkpoint blocking (ICB) therapy critically relies on the killing of melanoma cells by CD8$^+$ T cells, becoming activated upon recognition of tumor antigens presented by HLA class I (HLA-I) surface molecules. Patient-derived melanoma cells can escape from cytotoxic T cell effector functions by loss of HLA-I surface expression due to the silencing of HLA-I antigen processing and presentation machinery (APM) genes.

Material and Methods Seeking for a strategy to restore HLA-I expression, we transfected melanoma cells obtained from distinct patient metastasis with synthetic short double stranded RNA (3pRNA), an activating ligand of the cytosolic innate pattern recognition receptor RIG-I. 3pRNA-transfected melanoma cells were analyzed for HLA-I surface expression by FACS analysis and gene expression of HLA-I APM components by qPCR. *In vivo* 3pRNA-transfected tumors were analyzed for HLA-I expression by immunohistochemistry staining. Furthermore, T cell activation after coincubation with 3pRNA-transfected melanoma cells was determined by IFNγ-ELISpot assay. The effect of combined 3pRNA and blocking anti-PD-1 antibody treatment on T cell activation was measured by intracellular cytokine staining and FACS analysis.

Results Activation of RIG-I by 3pRNA increased the expression of HLA-I APM components and strongly enhanced recognition of melanoma cells by autologous CD8$^+$ T cells. Based on these findings, we asked whether the combination of 3pRNA and blocking anti-PD-1 antibodies could improve anti-melanoma T cell responses in an anti-PD-1 non-responder patient model. Indeed, T cell activation by 3pRNA-transfected melanoma cells was significantly increased in the presence of anti-PD-1 antibodies. In line with the enhancement of anti-tumor T cell responses, we found an association of elevated RIG-I mRNA levels with prolonged patient survival in TCGA melanoma samples.

Conclusions In summary, this study demonstrates a beneficial effect of RIG-I activation on antigen presentation and T cell recognition of melanoma cells. Improved T cell responses by combined 3pRNA and anti-PD-1 treatment suggests that combinational therapy could be a strategy to overcome T cell resistance in melanoma.

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On Demand Talks

On Demand Talks: Tumor Microenvironment

O1 TUMOR LACTIC ACIDOSIS ALTERS DECISIVE T CELL ACTIVITIES

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10.1136/jitc-2020-ITOC7.6

Background Adoptive T cell therapy is a promising treatment strategy for tumor patients. However, when entering the tumor microenvironment (TME), T cells lose their effector function showing reduced degranulation and cytokine secretion. Besides T cell inhibition through checkpoint pathways (i.e. PD-1/L1, CTLA–4), suppressor cells (i.e. TAM, T_{reg}) and cytokines (i.e. IL–10, TGF, VEGF), various metabolites of the TME also counteract antitumoral activities. Among the latter, lactate and extracellular acidosis are byproducts of the cancer metabolism and commonly observed in high concentrations in

solid tumors. Previous experiments showed that tumor lactic acidosis selectively targets the signaling pathway including JNK/c-Jun and p38, resulting in inhibition of IFN-γ production. In contrast, granule exocytosis, which is regulated via the MEK1/ERK pathway, was moderately affected. Based on the contrasting effects on these two essential T cell effector activities, we investigated in more detail the effects of lactic acidosis on the killing process conducted by T cells.

Material and Methods Tumor cells and cytotoxic T cells were co-cultured in lactic acid or regular culture medium and analyzed for effector function by flow cytometry and cell-mediated cytotoxicity assays. Additionally, 'in-channel micropatterning' in combination with artificial intelligence (AI) aided image analysis was used to visualize and analyze T cell cytotoxicity and mobility on a single cell level. Usage of collagen-matrices allowed the observation of T cell activity in a physiological three-dimensional environment. Cell metabolism was analyzed by Seahorse technology.

Results In the presence of lactic acid, IFN-γ production was strongly inhibited, while degranulation was only moderately reduced. Detailed analysis of the different processes involved in T cell cytotoxicity revealed that T cell recognition of tumor cells resulted in less secretion of cytotoxins (perforin, granzyme B and granzyme A). Lytic activity against tumor cells was strongly reduced at low T cell to tumor cell ratio (1:2). This deficiency could be compensated by increasing the T cell to tumor cell ratio (10:1). Using live cell imaging we investigated underlying mechanisms that might explain how higher T cell to target cell ratios might overcome lactic acid inhibition. T cells in lactic acid covered less distance, they moved for longer time periods and made less contacts with tumor cells in comparison to T cells cultured in regular culture medium.

Conclusions Micropatterning and AI based image analysis allows for detailed assessment of the processes involved in T cell-mediated killing such as mobility, speed, directionality and attachment on target cells. Lactic acidosis is hampering T cell killing activity by reducing the T cell's capacity to find its target cell and attach to it. Repeated addition of T cells or neutralization of lactic acidosis in the TME are means to overcome these deficits and hold promise to improve the outcome of T cell-based immunotherapies.

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On Demand Talks: Precision Medicine Meets Immunotherapy (Immuno-Monitoring)

02 DIRECTLY LINKING SINGLE T CELL PHENOTYPE AND FUNCTION TO GENOTYPE

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T cell therapies for cancer treatment are challenging to develop because of the complex mechanisms and cell interactions that underly T cell-mediated tumor killing. Current technologies rely on correlating phenotype, function, and gene expression based on experiments performed on different populations of T cells because no one platform is able to assess cell surface marker expression, cytokine secretion, and tumor cell killing activity of the same T cell and recover this cell for downstream genomic analysis. Here we share two use cases - CAR-T cell functional screening and TCR sequence recovery following functional assay - that demonstrate how the T Cell Analysis Suite on the LightningTM optofluidic platform can be used to directly link T cell phenotype and function (IFNγ secretion and tumor cell killing) to genotype (TCR sequence recovery) at a single-cell level and on the same T cell, enabling deeper and more thorough characterization of how T cells mediate tumor cell death and potentially the development of more efficacious therapies.

Disclosure Information Y. Bronevetsky: A. Employment (full or part-time); Significant; Berkeley Lights Inc.

03 HIGH-DIMENSIONAL ANALYSIS OF TUMOR ARCHITECTURE PREDICTS CANCER IMMUNOTHERAPY RESPONSE

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10.1136/jitc-2020-ITOC7.8

Background Immunotherapies have induced long-lasting remissions in countless advanced-stage cancer patients, but many more patients have not benefitted. Therefore, novel predictive markers are needed to stratify patients before treatment and select those who will most likely benefit from immunotherapy, while avoiding potentially devastating adverse effects and high treatment costs for those who will not. We reasoned that thoroughly characterizing the architecture of the tumor microenvironment (TME) at the single-cell level by highly multiplexed tissue imaging should reveal novel spatial biomarkers of immunotherapy response.

Materials and Methods We used CODEX (CO-Detection by indEXing) highly multiplexed fluorescence microscopy to investigate the TME of cutaneous T cell lymphoma (CTCL) in samples from patients treated with pembrolizumab. 55 protein markers were visualized simultaneously using a tissue microarray of matched pre- and post-treatment skin biopsies from 7 pembrolizumab responders and 7 non-responders. After computational image processing and extraction of single-cell information, cell types were identified by unsupervised clustering followed by supervised curation, and cell-cell distances and 'cellular neighborhoods' were computed. We also performed RNA sequencing on laser-capture microdissected tissue microarray cores to extract cell-type specific gene expression profiles by CIBERSORTx analysis.

Results CODEX enabled the identification and characterization of malignant CD4+ tumor cells and reactive immune cells in the CTCL TME at the single-cell level, resulting in 21 different cell type clusters with spatial information. Cluster frequencies were not significantly different between responders and non-responders pre- and post-treatment. However, advanced computational analysis of the tumor architecture revealed cellular neighborhoods (CNs) that dynamically changed during

pembrolizumab therapy and were correlated with response. Effector-type CNs enriched in tumor-infiltrating CD4+ T cells and dendritic cells were significantly increased after treatment in responders. In contrast, a regulatory T cell-enriched CN was significantly increased in non-responders before and after therapy. Furthermore, a spatial signature of cell-cell distances between tumor cells and effector/regulatory immune cells predicted therapy outcome. In addition, CIBERSORTx analysis revealed that tumor cells in responders, but not in non-responders, increased their expression of immune-activating genes.

Conclusions High-dimensional spatial analysis of CTCL tumors revealed a pre-existing immunosuppressive state in pembrolizumab non-responders. Thorough analysis of the TME therefore enables the discovery of novel spatial biomarkers in a concept that accounts for both cell type information and higher-order tumor architecture. Combining highly multiplexed microscopy with CIBERSORTx allows for the discovery of novel, predictive spatial biomarkers of immunotherapy response and will pave the way for future studies that functionally address these cell types and their interactions.

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On Demand Talk: 'Lost in Translation'

04 MECHANISMS OF LUNG CANCER HYPER-PROGRESSION PROMOTED BY PD-1 IMMUNE CHECKPOINT BLOCKADE

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10.1136/jitc-2020-ITOC7.9

Background Immune checkpoint blockade (ICB) with antibodies against PD-1 or PD-L1 may provide therapeutic benefits in patients with non-small cell lung cancer (NSCLC). However, most tumours are resistant and cases of disease hyper-progression have also been reported.

Materials and Methods Genetically engineered mouse models of $Kras^{G12D}p53^{null}$ NSCLC were treated with cisplatin along with antibodies against angiopoietin-2/VEGFA, PD-1 and CSF1R. Tumour growth was monitored by micro-computed tomography and the tumour vasculature and immune cell infiltrates were assessed by immunofluorescence staining and flow cytometry.

Results Combined angiopoietin-2/VEGFA blockade by a bispecific antibody (A2V) modulated the vasculature and abated immunosuppressive macrophages while increasing $CD8^+$effector T cells in the tumours, achieving disease stabilization comparable or superior to cisplatin-based chemotherapy. However, these immunological responses were unexpectedly limited by the addition of a PD-1 antibody, which paradoxically enhanced progression of a fraction of the tumours through a mechanism involving regulatory T cells and macrophages. Elimination of tumour-associated macrophages with a CSF1R-blocking antibody induced NSCLC regression in combination with PD-1 blockade and cisplatin.

Conclusions The immune cell composition of the tumour determines the outcome of PD-1 blockade. In NSCLC, high infiltration of regulatory T cells and immunosuppressive macrophages may account for tumour hyper-progression upon ICB.

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On Demand Talk: Young Researcher Session

05 DECONSTRUCTION OF HAMPERED DENDRITIC CELL DEVELOPMENT BY MICRO-ENVIRONMENTAL CROSS-TALK IN AN ORGANOTYPIC HUMAN MELANOMA-IN-SKIN MODEL

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Background Immune suppressive conditions in the melanoma tumor microenvironment (TME) block dendritic cell (DC) development and lead to the accumulation of M2-like macrophages and myeloid-derived suppressor cells (MDSCs). This will effectively hamper T cell priming, recruitment, and effector functions, and so interfere with the efficacy of immunotherapy. Targeting tumor-mediated myeloid suppression represents an interesting therapeutic option to promote the immune attack on tumors. The preclinical human models currently used to study myeloid suppression often fail to reflect the complexity of the TME.

Materials and Methods To study the cross-talk between melanoma and stroma cells and assess its effect on DC differentiation, we therefore established an *in vitro* three-dimensional (3D) reconstructed organotypic human melanoma-in-skin (Mel-RhS) model, allowing the monitoring of tumor growth and progression for up to six weeks.

Results Significantly higher levels of immune suppressive cytokines (IL-10, M-CSF, VEGF, TGF-beta) were detected in the melanoma model, constructed with the BRAF- and PTEN-mutated SK-MEL-28 cell line, as compared to its control (without melanoma cells). Indeed, Mel-RhS culture supernatants interfered with monocyte-to-DC differentiation, leading to the development of M2-like macrophages with a distinct phenotype ($CD14^+CD1a^-BDCA3^+CD163^+CD16^+PDL1^+PDL2^+$), as established by polychromatic flowcytometry. Correlation matrix heatmap analysis identified IL-10, TGF-beta and M-

CSF as the main candidate mediators of this skewing of monocytes to an M2-like state. The use of specific neutralizing antibodies against each of these cytokines prevented the observed DC suppression to varying degrees. t-Distributed Stochastic Neighbor Embedding (t-SNE) identified specific shifts between monocytic subpopulations and modulated expression levels of associated surface markers. Neutralization of M-CSF reduced expression of BDCA3, PD-L2, and PD-L1, while increased CD16; whereas blocking TGF-beta led to a concerted reduction in CD14, CD163, PD-L1, and PD-L2 levels, but, unexpectedly, also of CD80. In contrast, IL-10 neutralization resulted in a decrease of all M2-related markers, while CD80 levels were upregulated. Interestingly, while the SK-MEL-28 cell line did not secrete detectable levels of IL-10 in traditional monolayer cultures, RNA *in situ* hybridization revealed *de novo* expression in Mel-RhS in melanoma cells, as well as in keratinocytes and fibroblasts.

Conclusions We conclude that the 3D configuration of the Mel-RhS model results in cross-talk between tumor and stroma, which allows for the delineation of immune suppressive pathways in the melanoma TME. Ultimately, this model could be used as a novel *in vitro* tool for preclinical testing of immune modulatory therapeutic agents.

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On Demand Talks: Combination Therapy

06 EXPRESSION OF ANTI-APOPTOTIC GENE CFLIP TO ENHANCE PERSISTENCE IN CAR T CELLS

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10.1136/jitc-2020-ITOC7.11

Background CAR T cell therapy has been successful for targeting blood cancers, but treatment of solid cancers has been limited due to the heterogenous nature of tumour-associated antigen expression on solid cancers, and the suppressive tumour microenvironment.[1] Another major obstacle to CAR T cell therapy is activation-induced cell death (AICD) of the CAR T cells.[2] In this study, we expressed the anti-apoptotic cellular FLICE-like inhibitory protein (c-FLIP short; c-FLIPs) together with the CAR construct to enhance CAR T cell persistence.[3]

Materials and Methods The anti-Her2 FRP5 CAR T construct with P2A-linked cFLIPs or cFLIPp43 was cloned into the Sleeping Beauty (SB) transposon vector (pSBtet-GP) or lentiviral vector, under the control of either a tet-on or a constitutive promoter. Construct expression was validated by qPCR and immunoblot analysis. CAR T cells were generated by SB transposition or lentiviral transduction of CD3/CD28 stimulated primary human T cells that were subsequently maintained with IL-2. Mitochondrial function and apoptosis were determined by resazurin assay and by flow cytometry using tetramethyl rhodamine (TMRE).

Results Overexpression of cFLIP (cFLIPp43 and cFLIPs) in pSBtet-GP demonstrated protection in both Jurkat T cell line and primary human T cells. pSBtet-GP was modified to overexpress cFLIPs and cFLIPp43 under tet-on promoter, with the anti-her2 CAR, GFP and rtTA under constitutive promoter. Transfer of the inducible cassette from the SB transposon to a lentiviral system resulted in a significant loss of tightness. Doxycycline treated CAR T cells showed only ~13-fold overexpression of cFLIPs or cFLIPp43 compared to untreated cells, and doxycycline significantly inhibited (approximately 30%) primary CAR T cell expansion. In contrast, constitutive expression of CAR-cFLIPs or cFLIPp43 construct gave a $>3 \times 10^5$-fold cFLIP overexpression, as compared to CAR-only control. While the transduction efficiency of CAR-only was around 70–80% control in primary T cells, this dropped to 20–25% when using the more genetically complex tet-on system.

Conclusions cFLIP protects T cells from Fas-induced apoptosis. The tet-on system demonstrates several drawbacks in the lentiviral system, including toxicity of the inducer drug (and/or squelching effects resulting in lowered viability), loss of responsiveness and lowered transduction frequencies. Therefore, a constitutive promoter system is preferred in lentiviral systems for the control of genes of interest within CAR T cells, while the SB transposon system may be preferred for tet-on control within CAR T cells.

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07 A BISPECIFIC VHH APPROACH TO LEVERAGE THE POTENT AND WIDELY APPLICABLE TUMOR CYTOLYTIC CAPACITY OF Vγ9Vδ2 T CELLS

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Vγ9Vδ2-T cells include a unique and potent subset of T cells which play an important role in tumor defense. Vγ9Vδ2-T cells recognize and can lyse butyrophilin 3A1-expressing target cells with elevated levels of non-peptide phosphoantigens (pAg), induced by cell stress or malignancy. To date, Vγ9Vδ2-T cell based cancer immunotherapeutic approaches were well tolerated and in some cases capable of inducing relevant clinical responses. In an effort to improve the efficacy and consistency of Vγ9Vδ2-T cell based cancer immunotherapy, we designed a bispecific VHH that binds to both Vγ9Vδ2-T cells and EGFR expressed by tumor cells and results in the target-specific activation of Vγ9Vδ2-T cells and subsequent lysis of colorectal cancer cell lines and primary colorectal cancer samples both in vitro and in an in vivo mouse xenograft model. Of note, tumor cell lysis was independent of mutations in KRAS and BRAF that are known to impair the efficacy of clinically registered anti-EGFR monoclonal antibodies as well

as common Vγ9Vδ2-T cell receptor sequence variations. In combination with the conserved monomorphic nature of the Vγ9Vδ2-TCR and the facile replacement of the tumor-specific VHH, this immunotherapeutic approach can in principle be applied to a large group of cancer types.

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O8 GEMCITABINE INDUCES PRO-APOPTOTIC BH3 ONLY PROTEINS AND SENSITIZES PANCREATIC DUCTAL ADENOCARCINOMA CELLS FOR RLH-TRIGGERED IMMUNOGENIC CELL DEATH

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Background Despite tremendous effort, the prognosis of patients with pancreatic ductal adenocarcinoma (PDAC) remains poor and therapy options are limited. Recent advances in chemotherapeutic schemes have increased the survival of PDAC patients by a few months only. So far, the success of immunotherapy seen in other cancer types could not be transferred to PDAC. Our group has demonstrated that single agent RIG-I-like helicase (RLH)-targeting immunotherapy induces an anti-tumoral immune response and improves survival in a PDAC mouse model dependent on the induction of immunogenic cell death. In addition, we and others were able to show that tumor cell death induction by RLH ligands is partially dependent on the induction of the pro-apoptotic BH3-only proteins PUMA and NOXA. In the current study we aim at improving therapy response using a combinatorial chemo-immunotherapy (CIT) approach.

Methods Tumor cell death induction by gemcitabine, oxaliplatin and 5-fluoruracil (5-FU) alone or in combination with RLH ligands was evaluated in the murine cell line Panc02. The induction of PUMA and NOXA was measured by real-time PCR. The capability of chemo-immunotherapy -induced tumor cell death to activate splenic $CD8a^+$dendritic cells (DC) as well as to induce antigen uptake and cross-presentation was investigated *in vitro*. Therapeutic efficacy was evaluated *in vivo* using an orthotopic PDAC mouse model.

Results Gemcitabine, oxaliplatin and 5-FU induced dose-dependent tumor cell death *in vitro*. however, only gemcitabine lead to an induction of the pro-apoptotic proteins PUMA and NOXA. Simultaneous treatment with gemcitabine and RLH-ligand increased cell death induction without affecting the cytokine secretion substantially. $CD8a^+$ DC activation upon RLH-therapy was not affected by chemotherapy. Of note, antigen uptake as well as T cell priming was increased by chemo-immunotherapy. Most importantly, the survival of orthotopic PDAC bearing mice was significantly prolonged in the chemo-immunotherapy group compared to single agent treatment.

Conclusions Gemcitabine treatment of PDAC induces PUMA and NOXA expression which leads to mitochondrial priming and sensitization towards RLH-induced cell death. chemo-immunotherapy increases the cross-presentation capability of DC *in vitro* and prolongs the survival of PDAC bearing mice. chemo-immunotherapy is therefore an attractive combinatorial therapeutic approach in PDAC.

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E-Poster Presentations

P01 Emerging concepts/novel agents

P01.01 A PHASE 1A/1B DOSE-ESCALATION STUDY OF INTRAVENOUSLY ADMINISTERED SB 11285 ALONE AND IN COMBINATION WITH NIVOLUMAB IN PATIENTS WITH ADVANCED SOLID TUMORS

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Background Immunotherapy has emerged as a transformative approach for the treatment of cancer. However, a significant percentage of patients are nonresponsive to these immunotherapies or experience disease relapse which highlights the need for new therapies. Recent work has highlighted a major role for Stimulator of Interferon Genes (**STING**) agonists in immunotherapy. Conceptually, the activation of the STING pathway in immune cells in the tumor microenvironment (TME) and tumor cells could result in the induction of innate and adaptive immunity and subsequent activation of cytotoxic T cells and NK cells for durable anti-tumor responses. SB 11285 is a novel agonist of STING pathway leading to the activation of tumor-resident APCs and priming of tumor antigen specific CD8+ T cells. In our preclinical studies using multiple tumor-derived cell lines, SB 11285 has been observed to cause the induction of cytokines, such as INF-b, INF-a, TNFa and others consistent with engagement of the STING target, as well as tumor cell death by STING-mediated apoptosis. SB 11285 reduced tumor volumes in multiple rodent tumor models when administered intravenously, intraperitoneally and intratumorally. Systemic administration could additionally facilitate trafficking of newly activated CD8+T cells from periphery into the tumor site. In addition, preclinical models indicate that survival and local tumor shrinkage were significantly enhanced when SB11285 was administered with anti-CTLA-4 or anti-PD-1 antibody, suggesting that SB 11285 can be administered with anti-PD-1 and anti-CTLA-4 antibody for synergistic activity. A multiple ascending dose, phase 1a/1b trial of SB11285 in multiple tumor types has been initiated and the objectives of this trial include determining a safe and efficacious dose of intravenous SB 11285 and a preliminary assessment of antitumor activity/efficacy as either monotherapy or in combination with nivolumab.

Materials and Methods This open-label, multicenter phase 1a/1b clinical trial (NCT04096638) aims to enroll approximately 110 patients in the dose escalation (Part 1) and expansion cohorts (Part 2). Part 1 of the trial is a dose escalation study with IV SB11285 monotherapy followed by combination with the checkpoint inhibitor nivolumab. Part 1 Dose Escalation of the study will evaluate ascending doses of intravenously administered SB 11285 with respect to dose-limiting toxicities (DLTs), maximum tolerated dose (MTD), recommended phase 2 dose (RP2D) and the pharmacokinetic (PK)/pharmacodynamic profile as monotherapy and in combination with nivolumab. SB 11285, with a starting dose of 0.3 μg/kg, will be administered as monotherapy weekly on Days 1, 8, 15, and 22 of repeated 28-day cycles in escalating doses and in combination with nivolumab administered on Q4W schedule. *Part 2 Expansion Cohorts* of the study will explore initial signs of efficacy in prespecified tumor types (such as Melanoma, HNSCC) using the recommended phase 2 dose (RP2D) of SB 11285 in combination with nivolumab. In addition, the biological effects of SB 11285 will be evaluated by changes in immune cell types and activation state, serum cytokines, and gene expression patterns indicative of activation of the immune compartment. The trial is being conducted at multiple sites in the U.S.

Disclosure Information A. Abbas: A. Employment (full or part-time); Modest; Spring Bank Pharmaceutical Inc. **J. Strauss:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Abbvie, Abbott Laboratories, Bristol-Myers Squibb, Intuitive Surgical, Johnson & Johnson, Merck. F. Consultant/Advisory Board; Modest; Tempus. Other; Modest; Dialectic Therapeutics. **F. Janku:** None. **R. Karim:** None. **A. Olszanski:** F. Consultant/Advisory Board; Modest; Bristol Myers Squibb. **J.J. Luke:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; All to institution for clinical trials unless noted) Abbvie, Bristol Myers Squibb, Medimmune, Necktar, Novartis, Merck, Leap, Incyte, Immunocore, Compugen, Corvus, Evil, Five Prime, Genentech, Immatic. F. Consultant/Advisory Board; Modest; Consultant: Akrevia, Algios, Array, Astellas,AstraZeneca, Bayer, Bristol Myers Squibb/Advisory Board:7 Hills, Actym, Alphamab Oncology, Mavu (now part of Abbvie), Pyxis, Spring Bank Pharma, Tempest. Other; Modest; Travel: Akrevia, Bayer, Bristol Myers Squibb, Reflexion, EMD Serono, Incyte, Janssen, Merck, Mersana, Novartis. **K. Leach:** A. Employment (full or part-time); Modest; Spring Bank Pharmaceuticals Inc. **R. Iyer:** A. Employment (full or part-time); Modest; Spring Bank Pharmaceuticals Inc.

P01.02 HLA CLASS-I AND CLASS-II RESTRICTED NEOANTIGEN LOADS PREDICT OVERALL SURVIVAL IN BREAST CANCER

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Background Tumors acquire numerous mutations during development and progression. These mutations give rise to neoantigens that can be recognized by T cells and generate antibodies. Tumor mutational burden (TMB) is correlated with, and has often been used as a surrogate of, neoantigen load, although that relationship is different depending on cancer types. Recent studies reported correlations between higher TMB and better overall survival after immune checkpoint blockade therapies in bladder, colorectal, head and neck, and lung cancers but not in breast cancer. On the other hand, the relationship between neoantigen load and survival has been controversial in literature. Higher neoantigen load has been linked to better overall survival in ovarian cancer and melanoma, but worse survival in multiple myeloma. Recently, no clear associations were found between neoantigen load and survival in 33 cancer types although only class-I restricted neoantigens were included.

Materials and Methods We developed a bioinformatics workflow, REAL-neo, for identification, quality control (QC), and prioritization of both class-I and class-II human leukocyte antigen (HLA) bound neoantigens that arise from tumor somatic single nucleotide mutations (SNM), small insertions and deletions (INDEL), and gene fusions. The correlations between TMB and neoantigen load per sample were calculated using Pearson Correlation Coefficient. TMB and neoantigen load comparisons between various groups were performed using Student's t-test. The survival analyses were performed using the Cox proportional hazards models while correcting for covariates.

Results We applied REAL-neo to 835 primary breast tumors in the Cancer Genome Atlas (TCGA) and performed comprehensive profiling and characterization of the predicted neoantigens. SNMs contributed to only 6.25% of the total neoantigens (# of class-I vs. class-II neoantigens = 1: 3.5); INDELs accounted for 57.17% of the total (class-I : class-II= 1:2), and gene fusions were responsible for 36.58% of the total (class-I : class-II = 1:2.2). TMB were positively correlated with total and each sub-categories of neoantigen load (class I: SNM: $r = 0.59$, $p < 2.2E-16$; INDEL: $r = 0.28$, $p < 2.2E-16$; gene fusion: $r = 0.26$, $p = 2.01E-11$; class II: SNM: $r = 0.47$, $p < 2.2E-16$; INDEL: $r = 0.16$, $p = 1.7E-05$; gene fusion: $r = 0.31$, $p = 4.37E-13$). The vast majority (99.75%) of the predicted neoantigens occurred in ≤1% of the cases and 83.76% were patient-specific found in one patient only. Tumors with somatic and germline functional mutations in BRCA1 or BRCA2 genes had higher TMB ($p = 2.76E-06$) and overall neoantigen load ($p = 0.009$). Lower HLA class-I and class-II restricted neoantigen loads from SNM and INDEL were found to predict worse overall survival independent of TMB, breast cancer subtypes, tumor infiltrating lymphocyte (TIL) levels, tumor stage, and age at diagnosis (class-I: HR = 1.81, $p = 0.04$; class-II: HR = 1.89, $p = 0.042$).

Conclusions Our study highlighted the importance of accurate and comprehensive neoantigen profiling and QC, and is the first to report the predictive value of neoantigen load for overall survival in breast cancer. This work was support by the State of Florida Cancer Center Grant, the bioinformatics program of Mayo Clinic Center for Individualized Medicine, and the Mayo Clinic inter-SPORE development grant.

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P01.03 VOC PATTERN RECOGNITION OF LUNG CANCER: A COMPARATIVE EVALUATION OF DIFFERENT DOG- AND ENOSE-BASED STRATEGIES USING DIFFERENT SAMPLING MATERIALS

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Background It has been reported that canine scent tests offer the possibility to screen for cancer. Assuming that breath samples can be collected with carrier materials, we tested the practicability of different carrier materials to be presented to dogs, and validated and compared results with an eNose. Moreover, we hypothesised that cancer detection ability of dogs differs according to their working experience.

Materials and Methods In a methodological approach two dog teams participated, one using experienced working dogs and the other ordinary household dogs to find discover which dogs were better qualified and the best training method. To find best carrier material for breath sampling we compared charcoal containing glass tubes with fleece masks. In a second validating part, experienced working dogs were trained with improved training strategies. For breath sampling two different, previously successfully tested fleece-based carrier materials were used: one was used with the dog team and both materials were compared with eNose.

Results In the first part of the study it was shown overall that experienced working dogs performed better to family dogs and the dogs achieved a sensitivity of 45–59% and a specificity of 45–69%. Charcoal based breath sample carrier materials did not qualify for detection of VOC by dogs. In the second part of the study, the dogs achieved a specificity of 83% and a sensitivity of 56%, but with considerable differences between individual dogs. The eNose provided a specificity of 97% for both fleece based carrier materials and a sensitivity of 89% for fleece filled glass tubes and 100% for earloop masks. Measurements of breath samples collected directly in respiratory bags as reference measurements achieved a sensitivity and specificity of 100%.

Conclusions Our data confirmed that diagnostic accuracy of dogs depended on the type of dog training and on the carrier materials. A comparison of breath samples analysis with an eNose achieved better results for both, sensitivity and specificity, than for dogs. The use of fleece masks or fleeces in glass tubes as a sampling material can be recommended as successful VOC carriers, encouraging their use for clinical screenings.

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P01.04 A SPATIALLY RESOLVED, HIGHLY MULTIPLEXED BIOMARKER ANALYSIS PIPELINE THAT BRIDGES THE DIVIDE BETWEEN DISCOVERY AND CLINICAL RESEARCH

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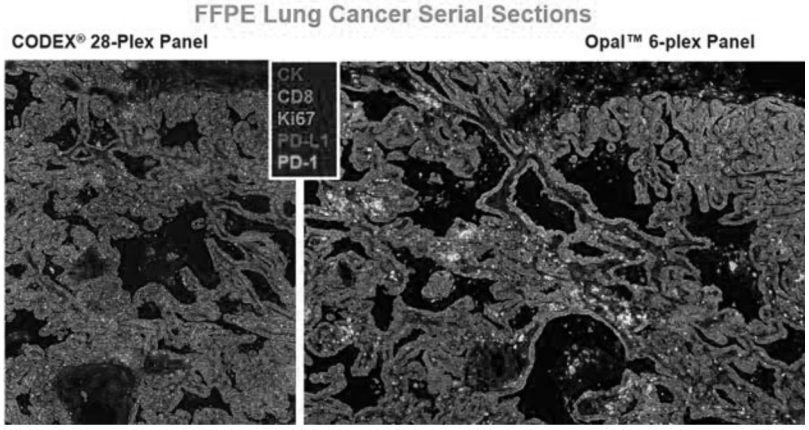
10.1136/jitc-2020-ITOC7.17

Background Multiplexed immunofluorescence (mIF) allows the visualization of multiple biomarkers in a single tumor tissue section, while at the same time preserving the spatial biology of the tumor microenvironment (TMI). CO-Detection by indEXing (CODEX®) and Phenoptics™ platforms are complementary mIF technologies that span the full spectrum of cancer research, from discovery to translational and clinical research. CODEX® is ultra-high plex and allows imaging of up to 40 antigens on a single tissue section with single-cell resolution. Phenoptics™ is an established mIF platform that enables high-throughput whole slide multispectral image acquisition and tissue interrogation with up to 8 markers plus DAPI. Here we present a study that compares shared sets of immune and tumor markers between the CODEX® and Phenoptics™ platforms. This cross-platform comparison provides a conceptual framework for researchers to translate biomarker signatures from discovery to high-throughput translational studies.

Materials and Methods Serial sections of human formalin-fixed paraffin embedded non-small cell lung cancer (NSCLC) and tonsils were analyzed. An initial screen with a 28-plex CODEX® antibody panel revealed multiple biomarkers of



Abstract P01.04 Figure 1

interest, including CK, CD8, Ki67, PD-L1 and PD-1; all of these biomarkers showed abundant expression in the TMI. Building on this result, we next developed a 6-plex Opal™ Phenotpics™ panel. This panel was screened and analyzed via high-throughput whole slide scanning of sample tissues. Image processing and data analysis were conducted similarly for both datasets so that repeatability and consistency of measurements could be established.

Results Both CODEX® and Phenoptics™ detected the same cell phenotypes and displayed similar frequencies of cells expressing CK, CD8, Ki67, PD-L1 and PD-1 in serial sections of tonsil and NSCLC tissues. These observations were consistent and cross-validated in data from CODEX® and Phenoptics™ platforms. Crucially, this means that the two approaches can be made analytically equivalent, and hence, that they can be used in conjunction with each other as research progresses along the continuum from discovery to translational and clinical research.

Conclusions Our cross-platform comparison provides a conceptual framework for biomarkers discovered on the CODEX® platform to be translated to the Phenoptics™ platform for high-throughput translational studies. The resulting comprehensive phenotyping and quantification data retain spatial context and provide unprecedented insight into tumor biology.

Disclosure Information O. Braubach: A. Employment (full or part-time); Significant; Akoya Biosciences. **M. Gallina:** A. Employment (full or part-time); Significant; Akoya Biosciences. **B. Remeniuk:** A. Employment (full or part-time); Significant; Akoya Biosciences. **C. Wang:** A. Employment (full or part-time); Significant; Akoya Biosciences. **N. Nikulina:** A. Employment (full or part-time); Significant; Akoya Biosciences. **R. Bashier:** A. Employment (full or part-time); Significant; Akoya Biosciences. **J. Kennedy-Darling:** A. Employment (full or part-time); Significant; Akoya Biosciences. **C. Hoyt:** A. Employment (full or part-time); Significant; Akoya Biosciences.

P01.05 DEVELOPMENT OF SIGNAL AMPLIFICATION FOR SPATIALLY-RESOLVED, HIGHLY MULTIPLEXED BIOMARKER ANALYSIS OF HUMAN TUMOR TISSUES

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Background Characterizing the complexities of the tumor microenvironment is fundamental to understanding cancer. Spatial relationships between infiltrating immune cells and the existing cellular matrix are now recognized as key determinants of tumor heterogeneity. Nevertheless, most available technologies for studying cells within the context of their tissue microenvironment, like traditional immunofluorescence (IF) and immunohistochemistry (IHC), are limited—allowing the visualization of only a few markers at a time.

Materials and Methods CO-Detection by indEXing (CODEX®) technology has overcome this limitation through a DNA-based labeling strategy, involving the sequential addition and removal of dye-labeled oligonucleotide reporters to antibodies equipped with complementary oligonucleotide tags. In this manner, it is possible to visualize tens of antibodies in the same tissue, in situ and at cellular resolution. Additionally, CODEX® interfaces with existing inverted microscopes and provides a cost-effective, fully automated platform for ultra-high plex immunofluorescence imaging. We have expanded the CODEX® platform to include Tyramide Signal Amplification of weak fluorescent signals, i.e. from low-expression biomarkers. This approach was tested with key biomarkers used in routine analyses of the tumor microenvironment, including PD-L1, PD-1 and FOXP3.

Results We demonstrate >50X amplification of PD-L1, PD-1 and FOXP3 signals when compared to control tissues. Moreover, we successfully included our amplification step in the CODEX® labeling/imaging workflow, so that it was possible to analyze amplified PD-L1, PD-1 and FOXP3 signals concurrently with a panel of 20+ additional antibodies. Analysis of our data also generated unique biological insights, including increased PD-L1 expression in T_{reg} cells and other tumor and stromal regions.

Conclusions Our findings demonstrate the feasibility of amplifying weak biomarker signals in the CODEX® workflow. Furthermore, our experiments were conducted on human formalin fixed paraffin embedded tumor tissues, thereby demonstrating the applicability of CODEX® analyses for clinical and translational research agendas.

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P01.06 SPATIALLY-RESOLVED, HIGHLY MULTIPLEXED BIOMARKER ANALYSIS OF CANCEROUS AND NORMAL HUMAN BREAST TISSUES

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Background Breast cancer has a high incidence rate and there is a need to develop new diagnostic tools and treatment regimens. Progress has, unfortunately, been slow and new technologies are urgently needed to generate a comprehensive understanding of breast cancer biology. Highly multiplexed imaging is an emerging tool that can help to unravel the complexities of the tumor microenvironment. This technology enables the detection of tens of biomarkers within a tissue specimen, and allows comprehensive cell phenotyping, biomarker quantification and spatial localization at cellular resolution. Such measurements can, in turn, provide insights into disease mechanisms and identify potential treatment targets. We demonstrate the development of a breast cancer specific CO-Detection by indEXing (CODEX®) panel that allows simultaneous in situ imaging of more than 30+ antibody markers.

Materials and Methods CODEX® relies on a DNA-based tagging approach, whereby antibodies are labeled with specific oligonucleotide tags (barcodes) and dye-oligonucleotides (reporters) are iteratively hybridized and dehybridized across

multiple cycles. This process is completely automated through the CODEX® instrument and readily deployable on commercially available fluorescence microscopy systems. Using a 30+ antibody CODEX® panel, we compared formalin-fixed paraffin embedded (FFPE) human breast cancer tissues at different stages of disease progression with normal breast tissues. Our antibody panel was designed to detect cancer cells as well as non-malignant cells in order to comprehensively survey the tumor microenvironment and normal control tissues. Data were analyzed using the CODEX® software suite to identify key cell types and analyze spatial associations.

Results Our analyses revealed more than 20 distinct cell types in human breast cancer and normal tissues. Cell populations, biomarker expression and cellular spatial distributions differed distinctly between cancerous and normal breast tissues. Differences were robust, repeatedly observed and indicative of altered cellular milieus in normal versus cancerous breast tissues.

Conclusions Collectively, these data establish CODEX® as a readily deployable and practical tool for spatially-resolved, highly multiplexed biomarker analysis of human FFPE samples.

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P01.07 TARGETING A MEMBRANE PROXIMAL EPITOPE ON MESOTHELIN INCREASES THE TUMORICIDAL ACTIVITY OF A BISPECIFIC ANTIBODY BLOCKING CD47 ON TUMOR CELLS

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Background Mesothelin (MSLN) is recognized as a relevant tumor-associated antigen for cancer immunotherapy, because of its overexpression on various solid tumors, including mesothelioma, pancreatic, lung, gastric and ovarian carcinoma. However, an anti-MSLN monoclonal antibody (mAb), amatuximab, has demonstrated only limited efficacy in clinical trials. It has been already demonstrated that the targeting of a membrane-distal domain of an antigen with a mAb is suboptimal at inducing Fc-related effector functions. As amatuximab targets a membrane-distal domain of MSLN, we investigated whether mAbs targeting different epitopes would bestow a better efficacy. Furthermore, in order to incorporate novel modalities to enhance tumor-killing, we have paired these MSLN targeting arms with an anti-CD47 arm to generate bispecific antibodies (bsAb). Indeed, the 'don't eat me signal' CD47 is a promising target in cancer and therapeutic blockade has recently showed clinical evidence of efficacy. Therefore, we investigated the contribution of a CD47 arm and the impact of the different anti-MSLN targeting arms on the tumoricidal activities of CD47xMSLN bsAbs.

Materials and Methods A panel of anti-MSLN mAbs and CD47xMSLN biAbs carrying the same anti-CD47 arm and different anti-MSLN arms were generated and characterized for their epitope specificity. Their tumor cell killing efficacy *in vitro* and *in vivo* was analyzed using cell-based assays, xenograft models and various MSLN+ human malignant cell lines originated from different tissues (e.g., lung, gastric and hepatic origin).

Results Our data revealed that all CD47xMSLN bsAbs, regardless of the recognized MSLN epitope, showed higher activity than the corresponding anti-MSLN mAbs in tumor-cell killing assays and demonstrated superior anti-tumor activity in a xenograft model. Targeting a membrane-proximal epitope rendered an anti-MSLN mAb more effective in mediating antibody-dependent cell-mediated cytotoxicity (ADCC) but did not optimize antibody dependent cellular phagocytosis (ADCP) activity. However, targeting the membrane-proximal epitope of MSLN afforded the CD47xMSLN bsAb enhanced ADCC and ADCP activity, resulting in superior activity *in vivo*. Mechanistically, engaging a MSLN membrane proximal region with a CD47-bsAb format not only enhanced FcγR-IIIA signaling but also interestingly disrupted more efficiently the CD47/SIRPα axis, resulting in optimized phagocytosis of tumor cells. Finally, we showed that treatment with CD47xMSLN bsAb targeting membrane proximal MSLN epitope induced an accumulation of myeloid cells and NK cells in the tumor microenvironment.

Conclusions This study demonstrated that when designing antibody-based molecules, the targeted region on a tumor-associated antigen needs to be carefully considered to ensure maximal effector function. In the context of MSLN-positive solid tumors, we showed that an approach targeting a membrane-proximal epitope coupled to a CD47-blocking arm afforded an improved ADCC and ADCP profile, translating into increased *in vivo* efficacy.

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P01.08 BEYOND PD-1: CHARACTERIZATION OF NEW CHECKPOINTS RESTRICTING FUNCTION OF CYTOTOXIC LYMPHOCYTES INFILTRATING HUMAN CARCINOMA

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Background T and NK cells from human renal cell carcinoma (RCC) are functionally non-responsive. Analysis of the TCR signaling cascade required for effector function identified that proximal signaling molecules were activated whereas activation of downstream ERK was blocked. Further investigation showed increased diacylglycerol kinase alpha (DGK-α) levels in T and NK cells from the RCC tumor microenvironment (TME). These cells were refractory to stimulation showing no degranulation or IFN-γ production. Using a small molecule DGK–α inhibitor (R59022), the function of tumor-infiltrating

lymphocytes was restored *ex vivo*. A correlation of high DGK-α and loss of function was also observed in an experimental mouse model of adoptive therapy where CAR T cells that had lost their activity after infiltrating into solid tumors were found to have increased DGK-α.[1] Blockade of the Programmed cell death protein 1 (PD-1) with monoclonal antibodies is used in the clinic enabling some patients to achieve tumor control. However, not all patients respond. DGK-α activity is positioned downstream of PD-1 and should, if overactive, curb T cell function even if PD-1 inhibition is released. Thus, we hypothesize that dual inhibition of PD-1 and DGK–α might be required to fully unleash the T cell's potential in the TME. Current DGK-α inhibitors are not suitable for clinical application. Therefore, we investigated alternative means using an RNA interference (RNAi) approach to target DGK-α alone as well as in combination with PD-1 in T and NK cells.
Material and Methods Knockdown is performed by RNAi using INTASYL™ compounds developed by Phio Pharmaceuticals. INTASYL™ compounds incorporate drug-like properties into the siRNA, resulting in enhanced uptake in the presence of serum with no need for further transfection reagents. Knockdown is analyzed by RT-qPCR and flow cytometry. Functional assays include cytotoxicity, degranulation and cytokine production in tumor mimicking environments.
Results A tumor mimicking *in vitro* system was developed which allows for the demonstration of functional restoration or prevention of functional loss of cell activity. Using T cell/tumor cell co–cultures at high tumor cell density, functional suppression could be induced in T and NK cells comparable to those observed in the TME. Testing of DGK-α targeting INTASYL™ compounds, silencing of DGK-α was observed in human U2OS osteosarcoma cells. Using a fluorescently labeled compound, highly efficient transfection of human primary immune cells was seen. Combinations of PD-1 and DGK-α targeting compounds are being tested and evaluated for synergism in experimental models.
Conclusions Strong activity of specific T and NK cells is necessary for tumor control. Dual targeting of PD-1 and DGK-α may be required to fully enable T and NK cell reactivity in the TME. Current DGK-α inhibitors do not exhibit the desirable pharmacokinetic/pharmacodynamic (PK/PD) properties for clinical development. The tested self-delivering RNAi technology represents a promising approach to targeting intracellular immune checkpoints such as DGK-α.

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P01.09 DUAL SIGNALLING PROTEIN 107 TRIGGERS INNATE AND ADAPTIVE IMMUNE RESPONSE TOWARDS TUMOUR CELLS

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Background Dual signalling protein 107 (DSP107) is a trimeric fusion protein consisting of the extracellular domains of human SIRPα and 4-1BBL. SIRPα binds to CD47, frequently overexpressed on cancer cells, and 41BBL binds to 41BB on activated T-cells. The SIRPα domain triggers the innate immune response by inhibiting the CD47/SIRPα 'don't eat me' signalling. It thus promotes phagocytosis of cancer cells by granulocytes, macrophages and dendritic cells. With its other side, 41BBL domain binds to pre-activated T cells and stimulates their expansion, cytokine production and cytolytic effector function. Our hypothesis is that augmented phagocytosis and improved co-localization of immune cells will lead to better antigen presentation towards activated T and B cells and the generation of memory T and B cells will be enforced. As result DSP107 might lead to immunity after rechallenge with the same tumour type.
Materials and Methods Primary phagocytes were incubated with stained tumour cells in presence or absence of DSP107 or/and therapeutic antibodies. Fluorescence microscopy measured uptake of tumour cells by macrophages. FACS identified primary granulocytes positive for CD11b staining and membrane dye. HT1080-41BB cells were mixed with HT1080-CD47 or HT1080-wt in presence of DSP107 and IL-8 release to supernatant was measured by ELISA. Further, primary T cells were co-cultured with αCD3Fc and fluorescent protein transduced carcinoma cells at different DSP107 concentrations.
Results The number of granulocytes that phagocyte tumour cells was increased in presence of DSP107. Further, DSP107 not only stimulated more macrophages to engulf tumour cells, but also the number of tumour cells that were taken up per phagocyte rose. Already enhanced phagocytosis of tumour cells by therapeutic antibodies (e.g. Cetuximab, Rituximab and Trastuzumab) was improved even further by DSP107. A model system showed that activation of the 41BB/41BBL axis by DSP107 was dependent on cross-linking via CD47 domain. This indicates low off-target T cell activation. Apart from the model system, DSP107 stimulated primary T cells in co-culture with carcinoma cells (transduced to express αCD3 and a fluorescent protein). Cytolytic activity against carcinoma cells was improved and outgrowth of tumour cells was reduced in a dose dependant manner.
Conclusions DSP107 blocks the CD47/SIRPα checkpoint resulting in enhanced tumour cell phagocytosis and stimulates the 41BB/41BBL axis leading to T cell mediated tumour cell killing. DSP107 is a novel bifunctional therapeutic that targets and activates both innate and adaptive anticancer immune responses. DSP107 is a first-in-class drug candidate that can

be used as a monotherapy or in combination with tumor-targeting monoclonal antibodies to trigger induction of anti-cancer immunity. DSP107 is currently tested in IND-enabling studies and clinical development is planned to commence in 2020.

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P01.10 IFNY SECRETION OF ADAPTIVE AND INNATE IMMUNE CELLS AS A PARAMETER TO DISPLAY LEUKAEMIA DERIVED DENDRITIC CELL (DC_{LEU}) MEDIATED IMMUNE RESPONSES IN AML

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Background Myeloid leukaemic blasts can be converted into leukaemia derived dendritic cells (DC_{leu}) with blastmodulatory Kit-I and Kit-M, which have the competence to regularly activate T and immunoreactive cells to gain anti-leukaemic activity or rather cytotoxicity. As innate and adaptive immune responses are notably promoted by the cytokine interferon gamma (IFNy), we hypothesised that the IFNy secretion could be a suitable parameter to display DC/DC_{leu} mediated immunologic activity and even anti-leukaemic cytotoxicity.

Materials and Methods DC/DC_{leu} were generated from leukaemic WB with Kit-I (GM-CSF + OK-432) and Kit-M (GM-CSF + PGE_1) and used to stimulate T cell enriched immunoreactive cells. Initiated anti-leukaemic cytotoxicity was investigated with a cytotoxicity fluorolysis assay (CTX). Initiated IFNy secretion of innate and adaptive immune cells (T cells, T^{CD4+} cells, T^{CD8+} cells, NK^{CD56+} cells, NK^{CD161+} cells, CIK^{CD56+} cells, CIK^{CD161+} cells and iNKT) was investigated with a cytokine secretion assay (CSA). In some cases IFNy production was additionally evaluated with an intracellular cytokine assay (ICA). Conclusively, the IFNy secretion of immunoreactive cells was correlated with the anti-leukaemic cytotoxicity.

Results Significant amounts of DC and DC_{leu} as well as migratory DC and DC_{leu} could be generated with Kit-I and Kit-M without induction of blast proliferation. T cell enriched immunoreactive cells stimulated with DC/DC_{leu} showed an increased anti-leukaemic cytotoxicity and an increased IFNy secretion of T, NK and CIK cells compared to control. Both the CSA and ICA yielded comparable amounts of IFNy positive innate and adaptive immune cells. The correlation between the IFNy secretion of immunoreactive cells and the anti-leukaemic cytotoxicity showed a positive relationship in T cells, T^{CD4+} cells, T^{CD8+} cells and NK^{CD56+} cells.

Conclusions We found blastmodulatory Kit-I and Kit-M competent to generate DC/DC_{leu} from leukaemic WB. Stimulation of T cell enriched immunoreactive cells with DC/DC_{leu} regularly resulted in an increased anti-leukaemic cytotoxicity and an increased IFNy dependent immunological activity of T, NK and CIK cells compared to control. Moreover the anti-leukaemic cytotoxicity positively correlated with the IFNy secretion in T cells, T^{CD4+} cells, T^{CD8+} cells, NK^{CD56+} cells. We therefore consider the IFNy secretion of innate and adaptive immune cells to be a suitable parameter to assess the efficacy of in vitro and potentially in vivo AML immunotherapy. The CSA in this regard proved to be a convenient and reproducible technique to detect and phenotypically characterise IFNy secreting cells of the innate and adaptive immune system.

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P01.11 ROLE OF EXOSOMES AS PROMOTORS OR BIOMARKERS TO STUDY ACTIVATION OF LEUKEMIA-DERIVED DENDRITIC CELLS (DCLEU)-MEDIATED ANTILEUKEMIC ACTIVATION OF ADAPTIVE AND INNATE IMMUNE-REACTIVE CELLS AGAINST AML-BLASTS

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Background Antileukemic responses of immune reactive cells in AML-patients need to be improved. Combinations of blast-modulatory kitM (GM-CSF+PGE1) (vs control) convert myeloid blasts into dendritic cells of leukemic origin (DCleu), that effectively activate immune-cells against leukemic blasts. Exosomes are small (30–150 nm) membranous vesicles of endocytic origin produced by all cells under physiological and pathological conditions. Their involvement in nearly all aspects of malignant transformation has generated much interest in their biology, mechanisms responsible for information transfer and their role in immune-surveillance as well as -escape.Exosomes secreted by dendritic cells (DCs) have been shown to allow efficient activation of T lymphocytes, displaying potential as promoters of adaptive immune responses.

Materials and Methods 1)DC/DCleu-culture of blast containing AML patients' whole blood (WB) (n=10) and of healthy volunteers(n=8) with kits, T-cell enriched mixed lymphocyte culture (MLC) with kit- vs un-treated WB, functional blast-cytotoxicity and, leukemia-specificity assays (Degranulation/intracellular cytokine-assays), Flowcytometric evaluation of blast-,DC- and lymphocyte composition before or after cultures. 2)Exosomes were isolated by immunoaffinity from serum, DC- and MLC-culture supernatants of 3 AML patients and 3 healthy volunteers. Exosomes were negatively stained and characterized by transmission electron microscopy (TEM). Fluorescence nanoparticle tracking analysis (fNTA) was performed to determine exosomal size and -concentration. Obtained results were compared in AML and healthy volunteers.

Results Addition of kitM to blast-containing WB significantly increased frequencies of mature DC/DCleu and their subtypes compared to untreated WB without induction of blasts' proliferation. Immune monitoring showed a continuous increase ofactivated/proliferating cells of the adaptive and innate immune system after Tcell-enriched MLC using kitM

pretreated vs -untreated WB, suggesting a production/activation of (potentially leukemia-specific) cells after kit-stimulation. Moreover kit-pretreated WB regularly and significantly improved provision, activation as well as antileukemic and leukemia-specifically directed immune reactive cells after MLC. TEM showed exosome-like structures with a typically cup-shaped appearance without any differences between healthy and AML samples. fNTA revealed average vesicle sizes of 177 ±23 nm (healthy) and 178±17 nm (AML). Higher levels of EVs were detectable in AML samples compared to healthy controls in serum and after DC-culture, but lower levels after MLC independent of culture conditions.Interestingly, the number of EVs increased during cultivation of DC of AML and healthy samples, but not in AML-derived MLC samples.

Conclusions We will provide data in AML patients and healthy volunteers about a potential role of DCs- and MLC-derived exosomes as biomarkers in immune responses, malignant progression or as potential therapeutic targets for AML patients.

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P01.12 IMPACT OF COMPLEMENTARY SUBSTANCES ON IMMUNE CELL ACTIVITY

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10.1136/jitc-2020-ITOC7.25

Background Natural substances and micronutrients are more and more included in anti-cancer therapy. However, benefit and harm both are reported from one and the same substance. This emphasizes the urgent need for the systematic analysis of a personalized approach which patient will profit from which substance.

Materials and Methods Risk analysis was performed using PBMCs isolated from patients diagnosed with advanced solid cancer. Benefit was analyzed using 3D-microtumors directly prepared from individual patient tumors. Blood cells and cancer cells were treated with different natural substances, namely curcumin, artesunate and vitamine C, as single agents and in combination therapy with guideline-directed drugs for 72h. Impact on cell metabolic activity was measured with the CellTiter Glo assay. The cell phenotype was described by FACS analysis.

Results In 80% of the patients natural substances induced a slight (mean: 10.7%, range: 2.3–17.7%) metabolic inhibition of the immune cells, which was minor in comparison to the strong immunotoxicity of chemotherapeutic drugs (e.g. 5-FU, mean: 33.5%; Gemcitabine: 67.2%). Contrary, 20% of the patients revealed a stimulatory effect on PBMC depending on the basic activity and the exhaustion of the immune cells. Combination therapy revealed that natural substances were able to reduce (mean: 16.4%, range: 5.2–42.8%) immunotoxicity mediated by chemotherapy. Analysis of the 3D-microtumors indicated that natural substances can mediate an anticancer effect, which was most obvious in relapsed tumors heavily pretreated with chemotherapeutic drugs. In addition, natural substances were identified as chemosensitizer. For example, curcumin was found to increase efficacy of Mitomycin C in breast cancer, Bicalutamid in prostate cancer and 5-FU combined with Cisplatin in gastric cancer.

Conclusions Complementary substances have a different effect depending on dosing, timing, cell type and cell characteristics. Therefore preclinical testing is required to identify the most effective complementary substances for the individual cancer patient analyzing both immune cells and cancer cells.

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P01.13 MERTK SIGNALING IS CRITICAL FOR T CELL PROLIFERATION AND MEMORY

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10.1136/jitc-2020-ITOC7.26

Background Overexpression of TAM receptors, including MERTK, in some cancers are integral for chemoresistance, proliferation and metastasis.[1] Our group has previously demonstrated that T cells also express MERTK and engagement of MERTK signaling is responsible for increased proliferation, functional capacity and metabolic fitness.[2] It is therefore important to further study the effect of MERTK inhibition on T cell function in the context of cancer treatments where MERTK inhibitors may play a role. Here we provide evidence that MERTK inhibition impacts greatly on T cell proliferation, specifically reducing phosphorylated mTOR. We have also demonstrated that MERTK expression is increased on CD8 central memory subsets during longterm expansion providing evidence that this signaling pathway may be important for sustaining T memory responses.

Materials and Methods Flow cytometric analysis was used to investigate the effect of titration of MERTK small molecule inhibitor UNC2025 on healthy donor T cells activated with CD3/CD28 dynabeads. Cell trace dye was used to track proliferation of CD4 and CD8 T cells along with markers of memory differentiation (CCR7 and CD45RO), activation (CD137) and function (IFNy, Tnfa and IL-2). MERTK signaling was assessed using phospho flow cytometric methodology of phosphorylated mTOR, AKT, ERK1/2, p38-MAPK and STAT5. Long term cultures of donor T cells of up to 28 days were investigated for MERTK expression alongside memory differentiation.

Results We demonstrated that moderate concentrations of MERTK inhibitor reduced proliferation of activated T cells. Despite inhibition of cell division, cell size still increased 2 fold compared to resting cells and cell viability remained unchanged. Additionally, the proportion of central memory to effector memory populations and intracellular cytokine production was not impacted. Analysis of molecules involved in MERTK signaling revealed that phosphorylated mTOR was significantly modulated following the addition of MERTK inhibitor. Long term culture of CD8 T cells demonstrated MERTK was significantly increased following early and late re-stimulation, and expression of MERTK was strongly associated with central memory subsets.

Conclusions Our results demonstrate that inhibition of MERTK signaling on T cells reduces cell division where mTOR is significantly impacted. Despite this, other functional aspects, such as intracellular cytokine production remain

unchanged. Therefore, interruption of MERTK signaling on T cells has a specific effect on cell division rather than cytotoxic function on a cell by cell basis. This has potential ramifications on the use of MERTK inhibitors to treat tumors where the ability to form substantial cytotoxic T cell populations might be reduced. In addition, increased MERTK expression on central memory subsets during long term culture suggests this signaling pathway could be critical for generating memory pools of T cells and provide new avenues for the improvement of adoptive T cell therapy protocols.

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Disclosure Information R.M. Powell: None. **M.J.W. Peeters:** None. **A. Rachbech:** None. **P.T. Straten:** None.

P01.14 EXCESSIVE BIOLOGICAL AGEING OF CIRCULATING NEUTROPHILS IN CANCER PROMOTES TUMOR PROGRESSION

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Background Beyond their well-established role in host defense, neutrophils are increasingly recognized to contribute to the pathogenesis of malignant tumors. Recently, ageing of mature neutrophils in the systemic circulation has been identified to be critical for these immune cells to properly unfold their anti-infectious properties. The role of neutrophil ageing in cancer is still unknown.

Material and Methods Employing syngeneic mouse models of head and neck squamous cell carcinoma (cell line SCC VII) and breast cancer (cell line 4T1), cytokine expression (by multiplex ELISA), neutrophil trafficking (by multi-channel *in vivo* microscopy and flow cytometry), and neutrophil function (*in vitro* assays) were analyzed.

Results Here, we show that signals released during early tumor growth promote excessive biological ageing of circulating neutrophils as indicated by age-related changes in their molecular repertoire. These events facilitate the accumulation of these highly reactive immune cells in malignant lesions and endow them with potent pro-tumorigenic functions. In particular, excessively aged neutrophils release neutrophil elastase which, in turn, stimulates the proliferation of cancer cells. Counteracting accelerated biological ageing of circulating neutrophils by blocking the chemokine receptor CXCR2 effectively suppressed tumor growth.

Conclusions Our experimental data uncover a potent self-sustaining mechanism of malignant tumors in fostering pro-tumorigenic phenotypic and functional changes in circulating neutrophils, thus supporting tumor progression. Interference with this aberrant process might provide a novel, already pharmacologically targetable strategy for cancer therapy. *This study was supported by Deutsche Forschungsgemeinschaft (DFG), Sonderforschungsbereich (SFB) 914.*

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P01.15 PERSONALIZED COMBINATION OF NEOADJUVANT DOMATINOSTAT, NIVOLUMAB (NIVO) AND IPILIMUMAB (IPI) IN MACROSCOPIC STAGE III MELANOMA PATIENTS STRATIFIED ACCORDING TO INTERFERON-GAMMA (IFN-GAMMA) SIGNATURE – THE DONIMI STUDY

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Background The previous OpACIN and OpACIN-neo studies investigating neoadjuvant IPI plus NIVO have demonstrated high pathologic response rates (74–78%) and favorable long-term outcomes for patients (pts) with a pathological response; at 36 and 18 months follow up only 1/71 (1.4%) responders has relapsed. In contrast, pathological non-responders have a poor prognosis; 15/23 (65.2%) have relapsed so far. This emphasizes the need for baseline biomarkers predictive of non-response and new neoadjuvant treatment combinations for these pts. In our previous studies, baseline IFN-γ signature high pts were more likely to respond to IPI plus NIVO. The DONIMI study tests the combination of NIVO ± IPI combined with a class 1 histone deacetylase inhibitor, domatinostat (DOM), according to the pts IFN-γ signature. We have developed a neoadjuvant IFN-γ signature, based on the signature previously described by Ayers *et al*., that will be used for the first time to classify pts in this prospective trial.

Trial design This two-center investigator-initiated phase 1b study aims to assess the safety and feasibility of neoadjuvant NIVO ± DOM ± IPI in 45 stage III melanoma pts with macroscopic de-novo or recurrent disease. IFN-γ signature high pts (n=20) will be randomized (stratified by center) to Arm A (2 cycles NIVO 240 mg q3wk) or Arm B (2 cycles NIVO 240 mg q3wk + DOM 200 mg twice daily (BID), d1-14, q3wk). IFN-γ signature low pts (n=25) will be randomized to Arm C (2 cycles NIVO 240 mg q3wk + DOM 200 mg BID, d1-14, q3wk) or Arm D (2 cycles NIVO 240 mg q3wk + IPI 80 mg q3wk + DOM 200 mg once daily (OD), d1-14, q3wk). Based on safety data of the first 5 pts in arm D, the remaining pts will be treated with either a higher dosing scheme (200 mg BID, d1-14, q3wks), a lower dosing scheme (100 mg OD, d1-14, q3wks) or the same dosing scheme (200 mg OD, d1-14, q3wks). The primary endpoint is safety and feasibility. A treatment arm will be declared as not feasible if 2/5 or 3/10 patients cannot adhere to the planned time of surgery (week 6 ± 1week) due to treatment-related adverse events. Biopsies (week 0, 3), blood samples (week 0, 3, 6, 12) and feces (week 0, 3, 6) will be collected for translational research. To date, 7 patients have been enrolled.

Clinical trial information NCT04133948

Disclosure Information I.L.M. Reijers: None. **E.A. Rozeman:** None. **P. Dimitriadis:** None. **O. Krijgsman:** C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Modest; BMS. **L.J.W. Bosch:** None. **S. Cornelissen:** None. **J. Bouwman:** None. **J.M. Versluis:** None. **D.**

Rao: None. **B. van de Wiel:** None. **A.J. Spillane:** None. **R.A. Scolyer:** F. Consultant/Advisory Board; Modest; MSD, Neracare, Myriad, Novartis. **A.M. Menzies:** F. Consultant/Advisory Board; Modest; BMS, MSD Oncology, Novartis, Pierre Fabre, Roche. **A.C.J. van Akkooi:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Amgen, BMS, Novartis. F. Consultant/Advisory Board; Modest; Amgen, BMS, Novartis, MSD, Merck, Merck-Pfizer, 4SC. **G.V. Long:** F. Consultant/Advisory Board; Modest; Aduro, Amgen, BMS, MassArray, Pierre-Fabre, Novartis, Merck MSD, Roche. **C.U. Blank:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; BMS, Novartis, Nanostring. F. Consultant/Advisory Board; Modest; BMS, MSD, Roche, Novartis, GSK, AZ, Pfizer, Lilly, Genmab, Pierre Fabre.

P01.16 EFFECTS OF THE STAT3 INHIBITORS ON SENESCENT TUMOUR CELLS

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Background Cellular senescence is the process of cell proliferation arrest. Premature cellular senescence can be induced by chemotherapy, irradiation and, under certain circumstances, by cytokines. Senescent cells produce a number of secreted proteins and growth factors that may either stimulate or inhibit cell proliferation. One of the major cytokines that play role in regulation of cellular senescence is IL-6. IL-6/STAT3 signaling pathway represent decisive regulatory factors in cellular senescence. The objective of this study was to compare the effects of the STAT3 inhibitors on senescent and proliferative tumour cells. Further, the therapeutic potential of the STAT3 inhibitors was evaluated using murine tumour models.

Materials and Methods *In vitro*, as well as *in vivo* experiments were performed using TC-1 (model for HPV16-associated tumours) TRAMP-C2 (prostate cancer) cell lines. C57Bl/6NCrl mice, 7–8 weeks old, were obtained from Velaz (Prague, Czech Republic). Experimental protocols were approved by the Institutional Animal Care Committee of the Institute of Molecular Genetics (Prague, Czech Republic). STAT3 inhibitors, namely STATTIC, BP-102 (synthesised at the University of Hradec Kralove) and their derivatives were tested for their effects on tumour cells, such as cytotoxicity, ability to inhibit STAT3 phosphorylation, cell proliferation and tumour growth in syngeneic mice.

Results We have previously demonstrated that docetaxel-induced senescence in the TC-1 and TRAMP-C2 murine tumour cell lines, which was proved by *in vitro* (detection of increased p21 expression, positive beta-galactosidase staining, and the typical SASP capable to induce 'bystander' senescence), and *in vivo* experiments, using C57BL/6 mice [1]. Both TC-1 and TRAMP-C2 cells displayed elevated IL-6 secretion and activated STAT3 signaling pathway. Therefore, we tested efficacy of the STAT3 inhibitors on these cell lines. Cytotoxic and STAT3 phosphorylation inhibitory effects of the inhibitors were observed in both proliferating and senescent cells. Antitumor effects of selected inhibitors were evaluated.

Conclusions Collectively, STAT3 is an attractive target for therapeutic approaches in cancer treatment and we can assume that inhibition of the STAT3 pathway can be used for elimination of the pernicious effects of the senescent cells.

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P01.17 TIM-3/GALECTIN-9 PATHWAY CONTROLS THE ABILITY OF MALIGNANT CELLS TO ESCAPE HOST IMMUNE SURVEILLANCE. REGULATORY MECHANISMS AND THERAPEUTIC TARGETS

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Background Human cancer cells implement a variety of biochemical mechanisms which allow them to escape host immune surveillance resulting in disease progression. We have reported that the immune receptor Tim-3 and its natural ligand and possible trafficker galectin-9 determine the capability of human acute myeloid leukemia (AML) cells to evade cytotoxic immune attack.[1] Our further studies demonstrated that breast, colorectal and other human solid malignant tumour cells display high activity of this pathway[2] which can also be used for immune evasion. It is, however, important to understand the mechanisms which regulate the biochemical activity of Tim-3/galectin-9 pathway and expression of its components as well as the molecular basis of its capability to impair anti-cancer activity of cytotoxic lymphoid cells.

Materials and Methods In this study we used human cancer and non-malignant cell lines as well as primary human malignant tumour samples. We also used primary human T cells and natural killer (NK) cells. Western blot analysis, ELISA, quantitative real-time PCR, on-cell Western, immunohistochemistry, flow cytometry and biochemical assays were used as key instrumentals to conduct measurements.

Results We found that galectin-9 is used by human cancer cells to escape host immune surveillance. Cancer cells use various biochemical pathways to overexpress galectin-9. Regardless the biochemical background, transforming growth factor-beta (TGF-β) and transcription factor Smad-3 play crucial role in galectin-9 expression in human cancer cells. We identified the key receptors through which galectin-9 can then trigger killing of cytotoxic T lymphocytes and impairing of anti-cancer activity of natural killer cells.

Conclusions In this work, we report the biochemical mechanisms underlying overexpression of galectin-9 in human

malignant tumour cells and its differential effects on human cytotoxic lymphoid cells.

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P01.18 METABOLIC STATUS AND IMMUNE ACTIVATION INFLUENCE CLINICAL OUTCOMES IN PATIENTS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

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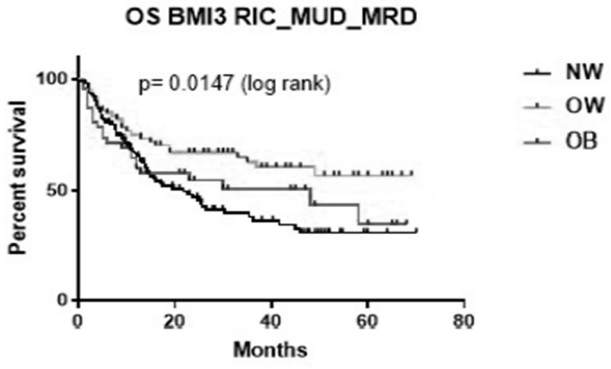
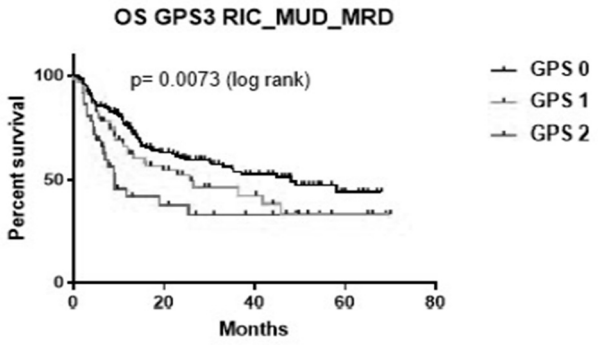
10.1136/jitc-2020-ITOC7.31

Background The nutritional status is an important factor contributing to non-relapse mortality for patients undergoing allogeneic hematopoietic stem cell transplantation (alloHSCT). In contrast to underweight, the role of overweight and obesity for alloHSCT outcomes is less well understood. This might be due to the use of the body-mass-index (BMI) as a classic measure of metabolic risk, which does not necessarily reflect body composition and visceral obesity. Importantly increased inflammation and malnutrition (Glasgow-Prognosis-Score, GPS) as well as increased neutrophil-to-lymphocyte ratios (NLR) have been described as adverse prognostic factors for a variety of solid tumors. In alloHSCT the GPS and NLR are ill defined so far. Here, we analyzed the impact of pretransplant GPS and d+100 NLR in correlation to the BMI on clinical outcomes following alloHSCT.

Materials and Methods Clinical data of consecutively treated patients between 2012 and 2017 at our transplant center were analyzed. From these cases only matched (10/10) related and unrelated donors and reduced intensity conditionings were included into the analysis. Based on BMI and GPS prior to conditioning we defined three groups respectively: normal weight (NW), overweight (OW), obese (OB); and GPS 0 (CRP<10 mg/dl, normal protein), GPS 1 (CRP >10 mg/dl, normal protein), GPS 2 (CRP >10 mg/dl, low protein). NLR at d+100 were also analyzed. We focused on survival and mortality until the data lock. Incidence rates of acute graft-versus-host disease (aGvHD) were determined until day+100.



Abstract P01.18 Figure 1



Abstract P01.18 Figure 2

Results From a total of 464 identified records, 265 cases were included into the analysis based on the inclusion criteria. Median overall survival in OB patients was doubled compared to NW (48 vs. 21.8 months; p=0.01) and in OW patients median overall survival was not reached (figure 1, *not included in the submitted abstract)*. Pretransplant GPS could also dissect survival curves with worst OS for patients with GPS 2: 9 (GPS 2) vs. 25.6 (GPS 1) vs. 48 (GPS 0) months, p=0.007). However, GPS values did not correlate with BMI (figure 2, *not included in the submitted abstract)*.

Increased d+100 NLR correlated non-significantly with poorer survival and increased relapse rates. There was a trend to increased clinically relevant aGvHD (≥II°) in GPS 2 individuals, not detectable according to BMI groupings.

Conclusions There is a need for better immunometabolic risk measures in patients before alloHSCT. Our data suggest that pretransplant GPS and NLR could be of value for risk estimations and further validation is warranted.

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P01.19 ABSTRACT WITHDRAWN

P01.20 TIM-3-GALECTIN-9 IMMUNOSUPPRESSIVE PATHWAY IN HUMAN LIQUID AND SOLID TUMOURS

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Background In recent years there has been increasing evidence highlighting biochemical pathways operated by human cancer cells, which allow them to escape host immune surveillance. Understanding the molecular basis of these immune evasion pathways and mechanisms underlying their biochemical regulation would allow development of fundamentally novel strategies of anti-cancer immunotherapy. This work is devoted to understanding the pathological role of Tim-3-galectin-9 immunosuppressive pathway.

Materials and Methods To complete this work we used human cancer and non-malignant cell lines as well as primary human malignant liquid (leukaemia) and solid tumour samples. We also used human primary natural killer (NK) cells and TALL-104 cytotoxic T cell line. Western blot analysis, on-cell and in-cell Western analysis, ELISA, quantitative real-time PCR, flow cytometry (including imaging flow cytometry) and biochemical enzyme activity were used as research tools.
Results We found that galectin-9 is highly expressed in human liquid (acute myeloid leukaemia (AML) and solid (breast, colorectal, brain etc.) tumour cells. G protein-coupled receptors of latrophilin family and their natural ligand fibronectin leucine rich transmembrane protein 3 (FLRT3) trigger externalisation/exocytosis, and, in some cases (e. g. AML), biosynthesis of galectin-9 and its receptor and possible trafficker Tim-3. Galectin-9 can be used to suppress anti-cancer immune responses by impairing cytotoxic activity of NK cells and killing T cells.
Conclusions We report the Tim-3-galectin-9 secretory pathway as one of the biochemical mechanisms operated by human cancer cells to escape host immune surveillance. Differential activities based on cell type of origin are discussed.
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P01.21 VALIDATION OF ROMO1 INHIBITORS AS THE MITOCHONDRIAL ROS ENHANCER FOR ANTICANCER DRUG DEVELOPMENT

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Background Chemotherapy in conjunction with surgical operations have been commonly used for the treatment of many tumors. However, a significant number of tumors fail respond to radiation therapy and/or chemotherapy because many forms of tumors appear to become less sensitive or resistant to radiation and anticancer drugs after consecutive treatments. Although extensive studies on the molecular mechanisms of resistance to chemo- and/or radiation therapy have been carried out, problems related to overcoming this resistance remain to be solved. Romo1 is a nuclear-encoded small transmembrane protein located in mitochondrial inner membrane. It is known to induce mitochondrial reactive oxygen species (ROS) production in response to various cellular stresses. For a decade, Romo1 has been studied in the context of mitochondrial ROS production, cancer cell invasion, inflammation, replicative senescence, and mitochondrial dynamics.
Materials and Methods We identified a Romo1 antagonist and tried to its efficacy as chemotherapy sensitizer using cancer cells and animal models.
Results A Romo1 antagonist can enhance the cellular levels of ROS, leading to tumor cell death. Its treatment induced the elevation of chemotherapy-induced oxidative damage of cancer cells. We also treated the Romo1 antagonist in combination with various chemotherapeutic agents.
Conclusions We suggest that Romo1 antagonist can enhance the cellular levels of ROS, leading to elevation of chemotherapy-induced oxidative damage of cancer cells. We also suggest that Romo1 is the new target to identify effective substances for development of chemotherapy sensitizer.

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P01.22 EXTENDING CAR T CELL THERAPY APPLICATIONS VIA DRUG INDUCIBLE CONTROL OF TRANSGENE EXPRESSION

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Background Adoptive transfer of chimeric antigen receptor (CAR)-modified T cells has emerged as a promising treatment modality for a broad range of cancers highlighted by the approval of Kymriah™ and Yescarta™ for the treatment of B cell malignancies. However, lack of control of CAR T cell function and consequent excessive inflammation in patients can result in severe side effects especially when targeting tumor-associated rather than tumor-specific antigens. Thus, temporal and tunable control of CAR activity is of major importance for the clinical translation of innovative CAR designs. While the activation of suicide switches results in the apoptotic elimination of the transferred cells, other strategies, e.g. anti-tag CARs or small molecule-gated CARs, enable the reversible control of CAR-mediated function at the protein level but are restricted to a particular CAR design. Focusing on the control of expression rather than CAR signaling, transcriptional regulators represent a versatile tool facilitating a wide range of CAR T cell applications.
Materials and Methods To maintain control over the infused CAR T cell product and mitigate risks for the patient, we describe here the development of an inducible switch system for the transcriptional regulation of transgene expression in primary, human T cells. Chemically regulated synthetic transcription factors composed of a zinc finger DNA-binding domain, an inducible control domain and a transcription activation domain were designed, screened for functionality, and evaluated in T cells regarding their potential to control CAR expression both *in vitro* and *in vivo*.
Results By screening, we identified a synthetic transcription factor, which shows high transcriptional output in T cells in the presence of a clinically relevant inducer drug and absence of background activity in the non-induced state. Using this system we were able to control the expression of a CAR recognizing the CD20 antigen present on B cells and B cell leukemic blasts. The addition of the inducer drug resulted in rapid expression of the anti-CD20 CAR on the T cell surface. Moreover, inducible anti-CD20 CAR T cells executed cytolytic activity against CD20 positive target cells and secreted cytokines upon stimulation *in vitro*. Effectivity in co-cultures was thereby comparable to T cells expressing the anti-CD20 CAR under a conventional constitutive promoter. Furthermore, we could fine-tune CAR activity by titrating the inducer concentration. By defining the time-point of induction, modulation of the onset of therapy was achieved. Upon inducer drug discontinuation, inducible CD20 CAR T cells lost CAR expression and concurrently all CAR-related functions, indicating that the 'on' and 'off' status can be tightly controlled by the administration of the drug. After pausing of CAR T cell-mediated activity, we could re-induce CAR expression suggesting complete reversibility of effector function. Finally, we were able to show that inducible CD20 CAR T cells mediate a

significant, strictly inducer-dependent antitumor activity in a well-established mouse model of B cell lymphoma.

Conclusions The zinc-finger-based transcriptional control system investigated in this study provides small molecule-inducible control over a therapeutically relevant anti-CD20 CAR in primary T cells in a time- and dose-dependent manner. The tight regulation of CAR expression will pave the way for safer cellular therapies.

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P01.23 CREATING A CELL-CULTURE BASED REPORTER SYSTEM FOR THE EVALUATION OF MOLECULAR SIGNALING MECHANISMS OF INHIBITORY CHIMERIC ANTIGEN RECEPTORS

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Background Adoptive transfer of T cells expressing chimeric antigen receptors (CARs) is a novel treatment option for patients with B-cell lineage derived cancers. Other cancer entities are less successfully targeted due to the lack of antigens that are expressed on cancer cells but not healthy tissue cells. One way to address this issue is the concept of inhibitory chimeric antigen receptors (iCARs) that deliver an inhibitory signal upon antigen encounter on off-target cells. The activating effect of CARs depends on a multitude of factors including expression levels, affinity, different signaling domains and steric effects. It has to be expected that co-expression of an iCAR would result in even more complexity. Consequently, there is a demand for a robust high-throughput cellular system to evaluate iCAR-formats.

Materials and Methods Our approach is based on a previously published Jurkat based triple parameter reporter cell (TPR) system. This setup allows for molecular monitoring of the T cell activation state by measuring fluorescent reporter gene expression via flow cytometry. Inhibitory effects of receptors are determined as the ratio of cellular geometric mean fluorescent intensity (gMFI) in the presence of the inhibitory ligand versus without inhibitory ligand.

Results To test if inhibitory receptors would measurably reduce reporter activation, PD-1 as a well-characterized inhibitory receptor was expressed on Jurkat TPRs. When PD-L1 was present during stimulation reporter activation was reduced by 26–34% proving feasibility of our approach. Intracellular domains of other inhibitory receptors including BTLA, ILT-2 and KIR2DL1 were evaluated. All three domains outperformed PD-1 in a series of experiments with a mean reduction of gMFI by 48–57%, 50–53% and 38–41% respectively. To assess if the reporter platform could be used to study downstream signaling pathways of inhibitory constructs we created a SHP-2 knock-out reporter cell line using the CRISPR/Cas9 gene editing technique. To simulate different degrees of activating signal strength, peptide-MHC complex recognizing activating receptors were created. The reporter activation correlated with the concentrations of peptide in the stimulation cultures. Co-expression of iCAR and CAR could be achieved using selection for two separate antibiotic resistance genes introduced into the respective vector. Preliminary experiments showed greatly reduced inhibitory efficacy of iCAR molecules due to an adhesion effect resulting from the high affinity extracellular domain of the iCARs that lead to tighter cell-cell contact and stronger stimulation through the CAR.

Conclusions We present a highly flexible and controllable Jurkat-based reporter cell platform for the thorough study of inhibitory signaling mechanisms. This project was supported by the Austrian Science Fund, FWF; Project P32411

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P01.24 THE SELECTIVE HDAC6 INHIBITOR ITF3756 INCREASES THE DIFFERENTIATION TO CENTRAL MEMORY T CELLS WITH REDUCED EXHAUSTION PHENOTYPE

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Background Central memory T cells show superior persistence and antitumor immunity compared to effector memory and effector T cells. T effector cells respond quickly to tumors, but they are terminally differentiated and undergo apoptosis upon killing activity. T memory differentiate rapidly into T effector cells and maintain a pool of cells that can continuously differentiate thus sustaining a more lasting response. In adoptive cell therapy (ACT), T cells infused into patients may have a limited time of activity if they are terminally differentiated, and may rapidly undergo exhaustion and apoptosis. The development of new strategies based on novel agents able to generate memory T cells ex-vivo is important for a successful clinical application of ACT.We have studied the effect of a potent and selective HDAC6 inhibitor, ITF3756, on CD8 T cells differentiation during an *in vitro* induced exhaustion process.

Materials and Methods To induce exhaustion purified human CD8+ cells were stimulated twice with anti-CD3/CD28 beads (1:2) during 5 days, with or without ITF3756 1μM or 2μM added at all times of stimulation. At day 3 and 5 the expression of exhaustion, memory and effector T cells markers were analyzed by flow cytometry. Cells were also collected at day 5 for genes expression analysis. Expression of exhaustion, T phenotype, metabolic pathway and inflammatory cytokines were investigated by qPCR. Paired two-tailed t-tests was used to determine statistical significance between control versus treatment group at day 3 and 5 in 10 different donors. P-values ≤ 0.05 were considered significant.

Results ITF3756 1μM increased significantly the T central memory phenotype (CD45RO+CD62L+CCR7+) and decreased significantly the T effector phenotype (CD45RO +CD62L-CCR7-). The expression of CD62L in T central memory cells was significantly increased in agreement with the high expression of this marker in naïve and memory T cells. ITF3756 treatment decreased significantly the expression of

exhaustion markers PD-1 and LAG-3. No effect was observed on TIM-3 expression. In agreement with the data obtained with protein analysis, treatment with ITF3756 reduced the mRNA level of Pd-1 and Lag-3. Gene expression of Tim-3 was also downmodulated, but this effect did not result in reduction of protein expression at the time of detection. ITF3756 reduced the expression of t-bet (Tbx21) driving T effector differentiation and increased genes related to T memory phenotype (Eomes, Lef-1 and albeit slightly, Tcf-7). T cell activation requires a metabolic reprogramming that supports highly proliferative phenotype and T effector differentiation. ITF3756 treatment decreased both Hif-1α and Glut-1 gene expression that are associated with TCR activation during the exhaustion process. T central memory cells produce less cytokines compared to T effector and effector memory cells. ITF3756 treatment decreased the genes expression of Il-2, Ifn-γ and Tnf-α. All these effects resulted dose dependent.

Conclusions The selective inhibitor of HDAC6 ITF3756 delays the terminal differentiation of CD8 T cells and increases the percentage of memory T cells with a reduced expression of exhaustion markers in vitro. These results are the basis to further explore the possible use of ITF3756 as a safe ex vivo treatment of CD8 T cells for adoptive cell transfer.

Disclosure Information C. Ripamonti: A. Employment (full or part-time); Significant; Italfarmaco SpA. **C. Steinkuhler:** A. Employment (full or part-time); Significant; Italfarmaco SpA. **G. Fossati:** A. Employment (full or part-time); Significant; Italfarmaco SpA.

P02 Microbiome and Immune System/ Immunotherapy

P02.01 PREDICTIVE IMPACT OF THE GUT MICROBIOTA ON TREATMENT RESPONSE TO CD19 SPECIFIC CAR T-CELLS

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Background High response rates (RR) have led to the approval of the CD19 specific CAR T-cell products Axicabtagene-Ciloleucel and Tisagenlecleucel for the treatment of refractory/relapsed B-cell precursor ALL (BCP-ALL) and Diffuse Large B-cell lymphoma (DLBCL). However, only a subgroup of patients achieves long-term remission. Additionally, most patients experience adverse effects such as cytokine release syndrome or neurotoxicity. Therefore, we need to better understand mechanisms of relapse and disease progression or toxicity to improve effectiveness of CD19 CAR T-cell therapy. As the gut microbiota plays an important role in modulating T-cell based immunotherapy, we hypothesize, that its signature also impacts clinical outcomes of CAR T-cell therapy.

Materials and Methods We are currently collecting and 16S rRNA sequencing fecal biospecimen from BCP-ALL and DLBCL patients before, during and after treatment with Axicabtagene-Ciloleucel and Tisagenlecleucel at the University Hospital of the LMU. Microbiota data are integrated into a patient-centered 'hospitalome' including onset and type of infection and of immunotoxicity, concomitant anti-infective and immunosuppressive agents as well as response to CAR T-cell therapy.

Results Preliminary data analysis revealed, that 4–14 days after CAR T-cell infusion the alpha diversity of the gut microbiome of each patient decreases drastically, whereas pre-lymphodepletion gut microbiota has a high diversity. Furthermore, the microbiota composition during the course of treatment changes as seen by beta diversity changes. In more detail, after CAR T-cell infusion, we observed instances of gut microbiota mono-domination with Enterococci or the genus Rikinella.

Conclusions The gut microbiome of patients being treated with CAR T cells undergoes large and diverse compositional changes. We currently explore how this microbiome heterogeneity relates to the distinct responses and immunotoxicity of patients after CAR T-cell therapy. Hence, this study will enable microbiome-based stratification of patients, in order to predict and improve patient outcome to this personalized T-cell recruiting immunotherapy approach.

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P02.02 GENERATING NEO- AND SELF-ANTIGEN SCREENING LIBRARIES FOR CLASS II HLA PRESENTATION

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Background The identification of neo-antigens presented by tumor cells is an essential tool for cancer prevention, diagnosis, and therapy. Current approaches frequently involve mass spectrometric analysis, but these workflows do not concomitantly identify the cognate T-cell receptor. Likewise, TCR functional screens are often limited to a subset of predicted neo-epitopes.

Materials and Methods Here, we present a new method for the generation of an un-biased antigen-presenting library. Due to the genomic instability of tumors, patient-specific libraries will be cloned using random primers, ensuring the cloning of tumor-specific transcribed regions. This approach will not only address class I presentation of intracellular tumor antigens, but is also designed to simultaneously screen for cross-presentation on class II MHC complexes by professional antigen-presenting cells, an increasingly important component of anti-tumor immune responses. To guarantee presentation of genetically encoded antigens on class II MHC complexes, a signal motif for chaperone-mediated autophagy (CMA) is introduced in front of the cDNA sequence. Furthermore, antigens will be processed by the intracellular machinery, avoiding potential restrictions on spliced peptides.

Conclusions Once established, these libraries can be exploited in high-throughput screens to functionally identify neo-antigens together with their corresponding T-cell receptor.

Disclosure Information V. Pinamonti: Other; Significant; Janssen. **N. Felix:** Other; Significant; Janssen. **J.M. Lindner:** Other; Significant; Janssen.

P02.03 MICROWAVE ABLATION ENHANCES TUMOR-SPECIFIC IMMUNE RESPONSE IN PATIENTS WITH HEPATOCELLULAR CARCINOMA

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Background Thermal ablative therapies, such as microwave ablation (MWA) or radiofrequency ablation (RFA), are standard treatments for HCC. In addition to the local tumor destruction, abscopal effects (a reduction of a tumor mass in areas that were not included in the thermal ablation) could be observed. These systemic effects may be mediated by anti-tumor immune response, which has been described for RFA. MWA is rapidly replacing RFA, but systemic immunostimulatory effects of MWA treatment have been poorly studied.

Materials and Methods Patients receiving MWA for localized HCC were included in this study. Effects of MWA on peripheral blood mononuclear cells (PBMC) of HCC patients treated with MWA were analyzed by multicolor flow cytometry. Tumor-specific immune responses against 7 shared tumor antigens were analyzed using peptide pools in 3-color Fluorospot assays (Interferon-y/Interleukin-5/Interleukin-10). The impact of type, density and localization of tumor-infiltrating lymphocytes was assessed by immunohistochemistry (IHC) of CD3, CD4, CD8, FoxP3, CD38 and CD20 and digital image analyses (Immunoscore) of tumor specimens in an additional cohort of patients who received combined surgical resection and thermal ablation.

Results While comprehensive flow cytometric analyses in sequential samples (day 0, 7 and 90) of a prospective patient cohort (n=23) demonstrated only moderate effects of MWA on circulating immune cell subsets, Fluorospot analyses revealed de novo or enhanced tumor-specific immune responses in 30% of these patients. This anti-tumor immune response was related to tumor control. Interferon-y and Interleukin-5 T cell responses against cancer testis antigens were more frequent in patients with a long-time remission (>12 months) after MWA (7/16) compared to patients suffering from an early relapse (0/13 patients). Presence of tumor-specific T cell response (Interferon-y and/or Interleukin-5) was associated to longer progression-free survival (15.0 vs. 10.0 months). Immunohistochemical analyses of resected tumor samples revealed that a high T cell infiltration in a second tumor lesion at the time of thermal ablation was associated with superior disease-free survival (37.4 vs. 13.1 months).

Conclusions Our data demonstrates remarkable immune-related effects of MWA in HCC patients. This study and provides additional evidence for a combination of thermal ablation and immunotherapy in this challenging disease.

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P02.04 ABSTRACT WITHDRAWN

P02.05 INVESTIGATION OF A SYNGENEIC A-PD-1 ANTIBODY TO TREAT MURINE 4T1 MAMMARY CARCINOMA

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Background Many cancers acquire mechanisms to evade immunosurveillance by activating immune checkpoint pathways, which suppress the antitumor immune responses. Monoclonal antibodies (ab's) targeting immune checkpoints, such as CTLA-4 and PD-1, have shown excellent results in several cancers and are currently being investigated in clinical trials for various malignancies. The clinically tested a-CTLA-4 (Ipilimumab) and a-PD-1 (Nivolumab and Pembrolizumab) ab's are fully human or humanized ab's, respectively. However, most studies conducted in mice utilize a xenogeneic a-PD-1 ab originating from rat, IgG2a RMP1-14 clone. This has been proposed to cause adverse effects in the commonly used 4T1 mammary carcinoma model of triple negative breast cancer (TNBC). Repeated administration of xenogeneic a-PD-1 ab's in this model results in fatal hypersensitivity reactions in tumor bearing mice, and unlike human TNBC, the 4T1 cell line is generally poorly responsive to immune checkpoint inhibitors. Recently, a semi-syngeneic recombinant a-PD-1 ab has been developed by transferring the variable regions of RMP1-14 onto a murine IgG1e3 constant region.

Materials and Methods Testing xenogeneic and semi-syngeneic a-PD-1 ab with and without a-CTLA-4 ab in BALB/c mice carrying 4T1 luciferase positive tumors.

Results In this study, we compared a semi-syngeneic recombinant a-PD-1 ab to the original xenogeneic RMP1-14 clone for treatment of luciferase positive 4T1 carcinomas. Surprisingly, the semi-syngeneic a-PD-1 ab was not able to circumvent the fatal hypersensitivity reactions. Still, the combination therapy of a-CTLA-4 and the semi-syngeneic a-PD-1 ab significantly reduced tumor volume in 4T1-luciferase tumor bearing mice compared to isotype control-treated mice already from day 16 post tumor inoculation (day 8 post treatment-initiation). In contrast, xenogeneic a-PD-1/a-CTLA-4 treated mice did not show significant difference from the control group until 24 days post tumor inoculation and never to the same degree. Furthermore, analysis of the T cell responses towards the murine tumor-associated antigen AH-1, revealed that treatment with syngeneic a-PD-1/a-CTLA-4 ab gave a significantly stronger $CD8^+$ T cell response over both control mice and mice treated with xenogeneic a-PD-1/a-CTLA-4 ab.

Conclusions These studies indicate that the semi-syngeneic a-PD-1 IgG1e3 ab might be a more efficient and translatable a-PD-1 ab for preclinical in vivo studies, which is important for the future investigation of immune checkpoint inhibitor therapy.

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P02.06 ABSTRACT WITHDRAWN

P03 Tumor Microenvironment

P03.01 PREVALENCE OF CD112R$^+$IMMUNE CELLS IN NORMAL LYMPHATIC TISSUES, INFLAMMATION AND THE CANCER MICROENVIRONMENT

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10.1136/jitc-2020-ITOC7.41

Background CD112R is an inhibitory immune checkpoint receptor and a putative target for novel immune therapies, but little is known about its molecular epidemiology in healthy and diseased tissues.

Materials and Methods To study the prevalence and expression level of CD112R$^+$ immune cells, we analyzed more than 200 samples of normal lymphatic, inflamed and cancerous tissues in a microenvironment tissue microarray format (4 mm tissue spot diameter) and large sections using fluorescent multiplex immunohistochemistry.

Results CD112R expression was detected at variable intensity levels in 47% of CD8$^+$ cytotoxic lymphocytes, 49% of CD4$^+$ T helper cells, 30% of FOXP3$^+$ regulatory T helper cells and in 25% of CD56$^+$ natural killer cells, but no expression was seen in CD11c$^+$ dendritic cells and CD68$^+$ macrophages. All analyzed compartments across normal and diseased tissues showed a small subset (CD8: 9±18%, CD4: 5±15%, FOXP3: 2±5%) of immune cells with supramaximal CD112R expression. The highest fraction of cells with supramaximal CD112R expression was found in the subset of CD8$^+$ cytotoxic T cells in the Peyer's patches of ileum (62%), the intergranuloma area of lymph node sarcoidosis (27%) and in ovarian cancer (37%). In cancerous tissues, the density and the fraction cytotoxic T cells with supramaximal CD112R expression was highly variable and ranged from 5% in bladder cancer to 3% in lung cancer and 36% in ovarian cancer. A high variability of the number of cells with supramaximal CD112R expression was also seen within every tumor entity.

Conclusions In summary, our analysis shows that CD112R expression is abundant in various subsets of immune cells but identifies a small fraction of cells with exceedingly high CD112R levels. The widespread occurrence of CD112R$^+$ cytotoxic T cells in the cancer microenvironment may suggest considerable opportunities for checkpoint inhibitors targeting CD112R.

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P03.02 SUPPRESSION OF T-CELL PROLIFERATION AND CYTOKINE RELEASE BY THE ADENOSINE AXIS ARE MEDIATED BY DIFFERENT MECHANISMS

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10.1136/jitc-2020-ITOC7.42

Background The so-called adenosine axis has emerged as a promising therapeutic target pathway as high adenosine levels in the tumor microenvironment contribute to the suppression of antitumor immune responses. The ectonucleotidases CD39 and CD73 act in concert to degrade extracellular immune-stimulating adenosine triphosphate (ATP) to immunosuppressive adenosine. According to the current model, subsequent suppression of effector immune cell function is caused by binding of adenosine to adenosine receptors like the A2a receptor (A2aR). The ectonucleotidases CD39 and CD73 as well as the A2aR have emerged as molecular targets within the adenosine axis with currently more than 20 clinical trials investigating antitumor effects of CD39-, CD73- or A2aR blockade. We aimed to perform a direct comparison of these targets with regard to their roles in regulating T-cell proliferation and IFN-γ secretion.

Materials and Methods CD39 and CD73 expression was suppressed using LNAplus^{TM} antisense oligonucleotides (ASOs). ASOs were synthesized as gapmers with flanking locked nucleic acids (LNA) to increase stability and affinity to the target RNA, leaving a central gap for recruitment of the RNA-degrading enzyme RNaseH I. Knockdown efficacy of ASOs on mRNA and protein level was investigated in primary human T cells. Furthermore, the effects of ATP, AMP and adenosine analogues on T–cell proliferation and IFN–γ secretion were investigated. A2aR was blocked using small molecule inhibitors that are currently under clinical investigation.

Results Treatment of human T cells with LNA-modified ASOs specific for human CD39 and CD73 resulted in potent target knockdown *in vitro* without the use of a transfection reagent. T-cell proliferation was reduced after addition of ATP to activated T cells that was completely reverted by ASO-mediated suppression of CD39 and/or CD73 expression but not A2aR inhibition. Adenosine analogues inhibited IFN–γ secretion of activated T cells, however, they did not suppress T-cell proliferation. Blockade of the adenosine kinase was able to revert the anti-proliferative effect of ATP degradation products, arguing for downstream metabolites of adenosine, but not A2aR signaling, being responsible for the suppression of T-cell proliferation.

Conclusions Cytokine secretion and proliferation of T cells might be differentially regulated by the adenosine axis. Adenosine might primarily affect cytokine secretion via A2aR signaling, whereas adenosine metabolites might especially impair proliferation of activated T cells independent from A2aR

signaling. Therefore, inhibition of CD39 and/or CD73 holds exceptional advantages over A2aR blockade as both, A2aR dependent and A2aR independent effects of ATP degradation products are targeted simultaneously.

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P03.03 ORGANIZATION, FUNCTION AND GENE EXPRESSION OF TERTIARY LYMPHOID STRUCTURES IN PDAC RESEMBLES LYMPHOID FOLLICLES IN SECONDARY LYMPHOID ORGANS

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Background Secondary lymphoid organs (SLO) are involved in induction and enhancement of anti-tumor immune responses on different tumor entities. Recent evidence suggests that anti-tumor immune responses may also be induced or enhanced in the tumor microenvironment in so called tertiary lymphoid structures (TLS). It is assumed that TLS represent a hotspot for T cell priming, B cell activation, and differentiation, leading to cellular and humoral anti-tumor immune response.

Methods FFPE-slides of 120 primary pancreatic ductal adenocarcinoma (PDAC) patients were immunohistochemically (IHC) stained for CD20, CD3, CD8 and HLA-ABC to analyze spatial distribution of tumor-infiltrating lymphocytes. 5-color immunofluorescence staining was performed to further investigate structural components of TLS in comparison to lymphoid follicles in SLOs. Microscope-based laser microdissection and Nanostring-base RNA expression analysis were used to compare gene expression in PDAC, TLS, SLOs and normal pancreatic tissue.

Results TLS were frequently detected in PDAC and were mainly localized along the invasive tumor margin. In less than 10% of the cases TLS were infiltrating the tumors. Interestingly, 20% of the patients had no TLS. Results of TLS will be correlated with clinical parameters, Immunoscore and immune escape mechanisms. 5-color Immunofluorescence staining revealed similar organization and function of TLS and SLO. Finally, gene expression analyzed by Nanostring revealed largely overlapping expression patterns in TLS and SLO.

Conclusions The results clearly demonstrate close similarities between SLO and TLS in terms of composition, distribution and gene expression Patterns.

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P03.04 APPLYING MULTISPECTRAL UNMIXING AND SPATIAL ANALYSES TO EXPLORE TUMOR HETEROGENEITY WITH A PRE-OPTIMIZED 7-COLOR IMMUNO-ONCOLOGY WORKFLOW

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Background The tumor microenvironment hosts a myriad of cellular interactions that influence tumor biology and patient outcomes. Multiplex immunofluorescence (mIF) provides the ability to investigate a large number of these interactions in a single tissue section, and has been shown to outperform other testing modalities for predicting response to immunotherapies.[1] Multispectral imaging (MSI) improves the capabilities of mIF by providing the ability to spectrally unmix fluorescence signals. This increases the number of markers that can be probed in the same scan and allows for separation of true immunofluorescence signals from tissue autofluorescence background. Here, we apply MSI to explore spatial interactions observed in lung cancer samples using an end-to-end translational workflow based on the Phenoptics™ platform. The workflow includes a pre-optimized 7-color staining panel kit along with a pre-configured analysis algorithm for cell phenotyping. Using tissue microarrays (TMA), we demonstrate the heterogeneity of spatial interactions observed among different lung cancer samples and the improved sensitivity of detection afforded by unmixing multispectral scans.

Materials and Methods Formalin-fixed paraffin-embedded (FFPE) lung cancer TMA contained 120 cores (1.5 mm diameter, US Biomax, Inc., Derwood, MD). The TMA was stained using the MOTiF™ PD-1/PD-L1 Panel: Auto Lung Cancer Kit and pre-optimized protocol for the Leica BOND RX™. Whole slide 7-color MOTiF multispectral scan was acquired on Vectra Polaris® using pre-defined parameters. Phenochart™ software was used to identify cores for analysis. Scans were unmixed and analyzed with inForm® software using a pre-configured algorithm tailored to the MOTiF™ PD-1/PD-L1 Panel kit. With this algorithm, cells are assigned phenotypes using intensity thresholds for CD8, PD-1, FoxP3, CD68, and PanCK signal levels, subject to pre-defined marker priority rules. The rules limit co-positivity to any combination of CD8, FoxP3, and PD-1, but no combinations of those markers with CD68 or PanCK, and no combination of CD68 with PanCK. When threshold levels generate excluded combinations, priority is given to calls for CD8/FoxP3/PD-1 over CD68, which in turn has priority over PanCK. To explore the dynamic range of PD-L1, it was assessed via expression level (signal intensity), not phenotyping. Spatial analyses and visualizations were performed in R [2] using the phenoptr and phenoptrReports packages[3], and custom scripts.

Results The pre-optimized Opal Polaris 7-Color PD-1/PD-L1 Lung Cancer Panel Kit was able to visualize the panel targets (PD-L1, PD-1, CD8, CD68, FoxP3, and Cytokeratin) across the variety of lung cancer samples in the TMA. Cell phenotyping and spatial analyses revealed core-to-core variations in cell densities and proximities among different markers.

Measurement of the dynamic range of PD-L1 expression across different cores also revealed the improved sensitivity in PD-L1 detection provided by unmixing.
Conclusions The end-to-end Phenoptics staining, imaging, unmixing, and spatial analysis workflow described here provides a robust and sensitive platform for exploring the immune landscape within the tumor microenvironment.
Disclosure Information V. Goubert: None.

P03.05 DEEP SPATIAL PROFILING OF THE IMMUNE LANDSCAPE OF MSI AND MSS COLORECTAL TUMORS

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Introduction In colorectal cancer (CRC) there have been many recent advances in immune related biomarkers that are both prognostic and predictive of response to immunotherapy. Microsatellite instability (MSI)/mismatch repair deficiency dMMR is present in 15–20% of CRCs and correlates with increased immunogenic mutations that often augment lymphocyte infiltration into the tumor microenvironment (TME). Additionally, location of tumor infiltrating T cells in two areas of the TME, the tumor center (CT) and invasive margin (IM) has also been shown to be prognostic and predictive of response to immunotherapy. Here we use multiplexed protein and RNA digital spatial profiling to elicit the immune landscape of MSI-MSS characterized CRC tumors.
Methods Forty-eight CRC tumors were analyzed for gene expression using the NanoString® nCounter® PanCancer IO 360™ Research Use Only (RUO) Gene Expression Panel and assessed for 48 cell typing and biological signatures, including MMR loss/MSI predictor and the Tumor Inflammation Signature (TIS). A subset of 18 CRC tumors (6 MSI-TIS-hi, 6 MSS-TIS-hi, 6 MSS-TIS-lo) was selected for analysis with the RUO GeoMx™ Digital Spatial Profiler (DSP) using 40 antibodies (human IO protein panel), or 84 RNA probes (human IO RNA panel). Selection of regions of interest (ROIs) in two locations, CT and IM were guided by staining with fluorescent markers (CD45, CD3, pan-CK, DNA). 300–600 μM diameter circle ROIs were selected, and in some cases segmented by pan-CK+/pan-CK-. For 2 immune hot samples contour profiling at the IM into stromal and tumor regions was performed using 1400+ RNA probes with NGS readout.
Summary Using whole tissue gene expression analysis, we determined the TIS and IO 360 signature scores for 48 CRC tumors using PanCancer IO 360 assay. 18 tumors within this cohort were selected based on TIS status to further dissect the location-dependent immune contexture of the TME. Protein DSP confirmed loss of dMMR markers (MSH2/MLH1) and identified an increased amount of potentially suppressive macrophages (CD163+PD-L1+) in MSI-TIS-hi versus MSS-TIS-hi tumors. Segmentation of ROIs based on tumor versus stroma (pan-CK±) identified samples with high proportions of tumor-invading TILs. Two MSI-TIS-hi profiled using probes against 1400+ mRNA targets confirmed protein results (CD163 in IM) and identified tumor-related signatures corresponding to the inside of the tumor (Cytokeratins, HER2/ERBB2, MET).
Conclusions Here we show the use of novel high-plex spatial profiling to profile location and pathways in the TME of MSI and MSS CRC tumors. These findings elicit unique biology related to the location and signaling of immune cells, which have the potential to unveil targets for therapeutic combinations.
Disclosure Information S.E. Church: A. Employment (full or part-time); Significant; NanoString Technologies. **J. Reeves:** A. Employment (full or part-time); Significant; NanoString Technologies. **D.R. Zollinger:** A. Employment (full or part-time); Significant; NanoString Technologies. **J. McKay-Fleisch:** A. Employment (full or part-time); Significant; NanoString Technologies. **A.J. Bahrami:** A. Employment (full or part-time); Significant; NanoString Technologies. **M. Holpert:** A. Employment (full or part-time); Significant; NanoString Technologies. **A.M. White:** A. Employment (full or part-time); Significant; NanoString Technologies. **M.D. Bailey:** A. Employment (full or part-time); Significant; NanoString Technologies. **C.R. Merritt:** A. Employment (full or part-time); Significant; NanoString Technologies. **M. Hoang:** A. Employment (full or part-time); Significant; NanoString Technologies. **S. Warren:** A. Employment (full or part-time); Significant; NanoString Technologies. **J.M. Beechem:** A. Employment (full or part-time); Significant; NanoString Technologies.

P03.06 PATTERN OF KI67$^+$EXPANDING CD8$^+$CYTOTOXIC T CELLS IN HEALTHY TISSUES, INFLAMMATION AND THE CANCER MICROENVIRONMENT

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Background Expansion of CD8$^+$ cytotoxic T lymphocytes is a prerequisite for anti-cancer immune activity. In the era of immune checkpoint therapy, profound knowledge of the dynamics of CD8$^+$ has regained considerable interest. However, systematically acquired data on CD8$^+$ proliferation in large sets of normal and diseased tissues are sparse.
Materials and Methods Here, we applied multiplex fluorescence immunohistochemistry to conventional large sections and tissue microarrays in order to quantitate Ki67$^+$CD8$^+$ cells in >20 different compartments of normal lymphoid tissues, 7 types of inflammatory diseases and 785 cancers.
Results In most normal lymphoid tissues (tonsil, lymph node, thymus, Peyer's patches, spleen, colon, appendix) the percentage of Ki67$^+$CD8$^+$ cells typically did not exceed 3%. The percentage of Ki67$^+$CD8$^+$ cells was markedly higher (45%) in the immune-active cortex of the thymus, however. In inflammatory conditions (including Hashimoto thyroiditis, Lichen sclerosus of the penis, sarcoidosis, sialadenitis, IgG4 pancreatitis, Crohn's disease and eczema), the percentage of Ki67$^+$CD8$^+$ cells was much more variable and often sharply higher than in normal tissues. It ranged from 0.5% in one patient with sialadenitis to 19% in the intraepithelial compartment of Crohn's disease. In 765 colorectal cancers, the fraction of Ki67 positive CD8$^+$ cytotoxic T cells ranged from 0 to 100% (mean: 20.6%). A high fraction of Ki67$^+$CD8$^+$ cells was significantly associated with microsatellite instability ($p<0.0001$), low pT stage ($p<0.0001$) and absence of nodal metastases ($p=0.0005$).
Conclusions In summary, our data show a variable increase of the fraction of proliferating CD8$^+$ T cells in cancers and in

inflammatory diseases as compared to healthy secondary lymphoid organs. The striking link with microsatellite instability and unfavorable tumor features suggest a potential clinical utility of assessing $Ki67^{+}CD8^{+}$ in colorectal cancer.

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P03.07 FAST AUTOMATED MICROFLUIDIC-BASED MULTIPLEXED IMMUNOFLUORESCENCE FOR TUMOR MICROENVIRONMENT ANALYSIS

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Background Immuno-oncology and targeted molecular therapies have acquired a central role in the treatment of multiple cancers. Consequently, high-throughput biomarker analysis and tumor immune profiling have seen an increased demand. Multiplexed immuno-assays are a powerful tool to address these needs, but still time- and resource-consuming. Our goal is to develop a fast and automated high-plex fluorescent immunostaining procedure, using a microfluidic-based device, that can be easily implemented as routine assay.

Materials and Methods Protocol optimization has been performed on FFPE sections of human tonsil. Slides were manually deparaffinized before being entirely processed (antigen-retrieval, staining, elution and counterstaining) by Lunaphore's autostainer, LabSat™. The OPAL® tyramide signal amplification (TSA) system was used as detection method. Signal analysis was done on Mantra® workstation. The 6-plex panel was composed of FoxP3, PD-L1, PD-1, CD68, CD8 and pan-CK, plus DAPI counterstaining. Protocols were subsequently transferred on NSCLC representative specimens and finally assessed on a TMA cohort.

Results Our platform allowed to reduce drastically the incubation times due to active transport of reagents across the tissue. Thereby, the automated 6-plex assay could be performed in less than 4h30min, within the timeframe of a single IHC standard assay. Protocol optimization resulted in high signal-to-background ratio for each marker and removal of previous step antibodies over 99%. LabSat™ also guaranteed remarkable signal uniformity, even over large tissue sections with less than 10% signal gradient over 1 cm. On NSCLC samples, the detected pattern and expression level for all six biomarkers were comparable to the standard chromogenic stainings performed with standard automated tissue stainer.

Conclusions LabSat™ autostainer enables multistaining runs in a timely manner, opening the perspective of rapid simultaneous detection of multiple markers in their morphological context on a routine-based approach. This versatile analysis tool can offer a better and more quantitative understanding of tumor heterogeneity and microenvironmental interactions, allowing advances in targeted therapy for lung cancer as well as broader spectrum of malignancies.

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P03.08 OMENTAL FAT IN OVARIAN CANCER POTENTIALLY INDUCES LYMPHANGIOGENESIS

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Background Ovarian cancer metastasis occurs by direct multifocal seeding in the peritoneum as well as by migration through the lymphatic system. High grade ovarian carcinoma patients present with distant metastases. Significant risk factors for the development of those are stage, grade, and lymph node involvement. An increase of the number of lymphatic vessels is shown in ovarian tumors and these vessels seem implicated in tumor progression. While the tropism of ovarian cancer cells for fat is well described, the potential impact of a fatty microenvironment on the dissemination of tumor cells via lymphatic vessels has, to our knowledge, never been investigated yet. In this study, we examined the effect of omental fat on lymphangiogenesis in ovarian carcinoma.

Materials and Methods To examine the effect of omental fat on lymphangiogenesis in OC we used a cohort of 80 human specimens. We analysed lymphatic vessels histologically with D2-40 and Lyve-1 markers. We also developed a healthy fat tissue explant culture model and treated explants with ascites of patient with OC before analysis. We analysed by fluorescence stainings the co expression of adipose derived stem cells (ASCs) and lymphatic markers in these explants.

Results We observed a higher density of tumor-associated vessels, especially lymphatic vessels in OC in contact with the omentum; mainly localized along the adipose tissue. We also measured a higher secretion of VEGF-C in tissues with fat compared to tissues without fat. Healthy fat tissues treated with ascites show an increase of number of ASCs, some of them express lymphatic markers such as D2-40 and Lyve-1. In a clinical trial of patients with OC treated by Bevacizumab, we observed a decrease of the number of lymphatic vessels in correlation with a decrease of the inflammation around the fat tissue.

Discussion We saw an increase in the number of lymphatic vessels in ovarian carcinoma infiltrating fat. These vessels are

principally distributed around the fat. We also observed an increase of proliferating ASC expressing lymphatic marker in fat explants treated with ascites. In a clinical trial of patients treated with Bevacizumab, we see a decrease of the lymphatic vessels. This decrease is linked with a decrease in the number of Inflammatory cells. These results together show that the fat tissue can play an important role in the lymphangiogenesis in the ovarian carcinoma. Furthermore, in the dissemination of metastasis through the body. We will next investigate the mechanisms underlying this phenomenon and try to understand all factors implicated in this process.

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P03.09 ABSTRACT WITHDRAWN

P03.10 PREVALENCE AND PROGNOSTIC ROLE OF FOXP3$^+$REGULATORY T LYMPHOCYTES IN CANCER. A TISSUE MICROARRAY STUDY ON >20'000 CANCERS

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Background Regulatory FoxP3$^+$ lymphocytes function as suppressors of T-cell activity. The clinical impact of high FoxP3$^+$ cell density in cancers is not fully understood, as some studies have linked high FoxP3$^+$ cell density to good prognosis and others to poor prognosis in tumor cohorts with associated clinical data. While some data suggest that these variable data are due to biological differences between tumor entities, it is also possible that methodological differences have caused these discrepancies. This study was undertaken to analyze the density of FoxP3$^+$ cells in various different cancer types by employing standardized methods.

Materials and Methods Tissue microarrays and large sections made from >20,000 prostate, breast, colorectal, ovarian, pancreatic, bladder and stomach cancers were analyzed together with various normal and inflamed tissues by conventional brightfield FoxP3 immunohistochemistry. Samples were also analyzed by fluorescent multiplex immunohistochemistry to assess the fraction of Ki67$^+$ FoxP3$^+$ cells.

Results Our results indeed suggested a variable role of FoxP3$^+$ cells in different tumor types. High FoxP3$^+$ density was linked to high Gleason grade ($p=0.0003$) and early biochemical recurrence ($p<0.0001$) in 16923 prostate cancers, but to low tumor stage ($p=0.027$) and prolonged survival ($p=0.0029$) in 1341 breast cancers, and to low tumor stage ($p<0.0001$) in 744 colorectal cancers. No significant associations were found to tumor phenotype in 549 ovarian, 574 pancreatic, 549 bladder and 346 stomach cancers. Multiplex fluorescence IHC analysis of FoxP3 and Ki67 revealed comparable fractions of proliferating FoxP3$^+$ cells in healthy tissues (average 12.3%, range 5.8–18.5%) and inflammatory conditions (average 7.6%, range 2.6–17.2%). Interestingly, the rate of Ki67$^+$FoxP3$^+$ cells was markedly higher in 36 bladder cancers (average 14.2%, range 0–49.3%) suggesting active expansion of FoxP3$^+$ cells in cancer.

Conclusions Our data demonstrate an inverse prognostic impact of the FoxP3$^+$ cell density in prostate and breast cancers. The increased proliferation rate of immune-regulatory FoxP3$^+$ cells in some bladder cancer is interesting in the light of the variable response of these tumors to immune checkpoint inhibitors.

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P03.11 EXPLORING TUMOR-INTRINSIC FACTORS REGULATING THE RECRUITMENT OF MYELOID-DERIVED SUPPRESSOR CELLS (MDSC) IN PANCREATIC DUCTAL ADENOCARCINOMA

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Background Pancreatic Ductal Adenocarcinoma (PDAC) has very poor 5-year overall survival rate. Despite the encouraging effect of immunotherapy in other cancer types, clinical benefit in PDAC patients remains limited. One of the reasons for the lack of success is the immunosuppressive tumor microenvironment (TME), which is maintained by myeloid-derived suppressor cells (MDSC) and tumor-associated macrophages. High MDSC infiltration is associated with a poor survival in PDAC patients. Our project aims at identifying tumor-driven chemokines that influence recruitment of MDSC and establishment of the immunosuppressive tumor microenvironment.

Materials and Methods 45 PDAC cell lines generated from spontaneous tumors of genetically-modified mice harboring the characteristic driver mutations *Kras^{G12D}* or *PIK3CA^{H1047R}* were analyzed for their expression levels of CXCL1, CCL2, G-CSF and GM-CSF by qRT-PCR. In order to study the relationship between the chemokine/cytokine profile and the immune cell infiltration, selected tumor cell lines were implanted orthotopically in C57BL6 mice. Three weeks after inoculation blood, spleen and tumor were isolated and organ specific immune cell infiltration was analyzed by flow cytometry. To further characterize tumor-secreted factors tumor conditioned medium was generated and the concentration of 33 chemokines was analyzed in a multiplex assay. The chemokine levels were correlated with migratory capacity of splenic MDSC measured in an *ex vivo* chemotaxis assay.

Results CXCL1 significantly enhanced migration of polymorphonuclear MDSC (PMN-MDSC) *in vitro*, while migration of monocytic MDSC (M-MDSC) was predominantly skewed towards CCL2. Three weeks after tumor inoculation, MDSC populations in blood and spleen were expanded. Most intriguingly, PDAC cell lines with high CXCL1 or CCL2 levels *in vitro* showed significantly enriched intratumoral accumulation of PMN-MDSC and M-MDSC, respectively, suggesting that tumor-intrinsic chemokine secretion and not factors from the tumor stroma determined MDSC infiltration. The *ex vivo* chemotaxis assays revealed additional factors that modulate migration of MDSC into the TME.

Conclusions The *in vitro* gene expression levels of individual chemokines (CXCL1 and CCL2) determines the MDSC infiltration *in vivo* into the TME. Targeting the chemokine-receptor axis of MDSC subpopulations could be a promising approach in the treatment of pancreatic cancer.

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P03.12 IMMUNOPHENOTYPING OF LIVER AND LUNG METASTASES IN COLORECTAL CANCER

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Background Immunotherapy is an attractive strategy for second-and further-line treatment of metastatic colorectal cancer (mCRC). However, currently immune checkpoint-inhibitors are limited to the small subgroup of dMMR-MSI-H patients. Therefore additional patient stratification markers for immunotherapy independent from the MSI-status are urgently required.

Materials and Methods In this study the immune infiltrate of 53 liver and 15 lung mCRC were immunhistochemically analysed and correlated with clinicopathological parametes related to the primary tumor and the metastatic lesion and the PD-L1 status. The CD3, CD8 and PD-1 infiltrate were quantitatively counted positive cells/mm² in three different topographic regions, namely invasion margin (IM), stromal (S) and intratumoral (IT). PD-L1 expression was semiquantitatively evaluated with the cut off > 1%. The statistical analyses were performed by the Fisher`s exact-Test (two-tailed).

Results In liver metastases (LM) a high immune infiltrate of CD3 IM, CD3 S, CD8 S and PD-1 S, significantly correlated with an advanced stage (pN1/2; cM1) of the primary tumor. Independent of the type of adjuvant chemotherapy, a significantly higher fraction of CD3+ and CD8+ cells was found at the invasion margin of LM. In contrast, neoadjuvant chemotherapy induced a reduction of PD-L1 expression. Interestingly, a high CD8 IT infiltrate and a high PD-L1 expression correlated with KRAS wildtype. In addition, a high CD8 IT infiltrate and a high PD-L1 expression were found in confined LM, defined as less than two segments and unilobular distribution. A high PD-L1 expression was accompanied by a strong infiltrate of CD3, CD8 and PD-1 positive cells. In contrast, the small cohort of lung metastases showed a significant correlation for a high CD8 S infiltrate and a PD-1 IM infiltrate with right-sided metastases. Additionally, a high PD-1 IM infiltrate could be seen after neoadjuvant chemotherapy in lung metastases.

Conclusions Chemotherapeutic treatment strategy might have an impact on subsequent immunotherapy. Combination of anti-EGFR inhibitors with immunotherapy and CD3/PD-L1 Bispecific antibodies are promising options to treat liver and lung metastasis of CRC.

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P03.13 AGE-INDUCED CHANGES IN ANTI-TUMOR IMMUNITY ALTER THE TUMOR IMMUNE INFILTRATE AND REDUCE RESPONSE TO IMMUNE-ONCOLOGY TREATMENTS

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Background Immuno-Oncology research relies heavily on murine syngeneic tumor models. However, whilst the median age for a cancer diagnosis is 65 years or older, for practical purposes the majority of preclinical studies are conducted in young mice, despite the fact that ageing has been shown to have a significant impact on the immune response.

Materials and Methods Using aged mice bearing CT26 tumors, we analysed how aging impacts the immune composition of the tumor, spleen and tumor-draining lymph nodes by flow cytometry.

Results We found many age-related changes between aged (60–72 weeks old) and young (6–8 weeks old) mice, such as a reduction in the naïve T cell population and a decreased CD8/Treg ratio in aged animals. Profiling of co-inhibitory and co-stimulatory receptor expression levels on immune cells in aged versus young mice also identified altered expression profiles in both the periphery and tumor. We hypothesised that these differences may contribute to impaired anti-cancer immune responses in aged mice. To investigate this, we compared the anti-tumor efficacy of immune checkpoint blockade (PD-L1 and CTLA-4) and T-cell costimulation (OX-40) in aged versus young mice. Our data demonstrate that aged mice retained their capacity to generate effective anti-tumor immune responses, albeit often attenuated when compared to the responses observed in young mice.

Conclusions These differences highlight the potential importance of age-related immunological changes in assessing and refining the translational insights gained from preclinical mouse models.

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P03.14 PRECLINICAL CASE STUDY: PATIENT-DERIVED HEAD AND NECK CANCER XENOGRAFT ON MICE HUMANIZED WITH AUTOLOGOUS IMMUNE CELLS, A MODEL FOR PERSONALIZED IMMUNO-ONCOLOGY RESEARCH

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Background The preclinical evaluation of novel immune modulators for cancer treatment remains a challenge, as models require both, engraftment of human tumor cells and a compatible human immune cells. In previous experiments, we have demonstrated, that we can use either peripheral blood mononuclear cells (PBMC) or hematopoietic stem cells (HSC) to establish a humanized immune system with functional T-, B-, and NK cells, monocytes, and dendritic cells. However these models are limited by rarely matching HLA isotypes between tumor and immune cells. In this case study, we established a patient-derived xenograft (PDX) model from a patient with Head and Neck squamous cell cancer (HNSCC). After engraftment of HNSCC PDX, patients PBMC were used to humanize mice. By this procedure we successfully generated a patient-specific human tumor-immune cell model in mice with 100% HLA-match. Model development included the comparison of PDX engraftment on mice with either HLA-matching or non HLA-matching PBMC's and purified T cells from different donors. Furthermore, these effects were investigated on humanized mice generated with HSC. Finally, we further validated the model by comparing treatment effects with the checkpoint inhibitor Nivolumab in the autologous immune cell PDX model with heterologous models.

Methods The HNSCC PDX was transplanted on NOG mice. After tumor engraftment mice were randomized in 6 groups, receiving PBMCs by i.v. transplantation either from the patient or from 5 well characterized donors (PDX patient PBMCs - 100% HLA matching, 5 donors with different HLA matching). In the last step, PDX were transplanted on humanized mice generated from 5 different HSC donors. Blood and tumor samples were analysed by FACS and IHC for immune cell infiltration and activation.

Results In the autologous huPBMC model, no interference with the proliferation of HNSCC PDX was seen. However, on mice humanized with donor PBMC's with a high HLA match, a strong stimulation of tumor proliferation compared to non-humanized mice was observed. On humanized mice, generated from 5 different HSC donors, HLA-matching seem to have a lower influence on engraftment. On mice humanized with PBMC from different donors, we observed a correlation of treatment effects with HLA match, with strong tumor growth inhibition in the mice with the best match. In the PDX tumors, infiltrating immune cells were detected by FACS and IHC analyses.

Conclusions We developed a humanized immune-PDX model enabling appropriate preclinical translational research on tumor immune biology and the evaluation of new therapies and combinations, as well as the identification and validation of biomarkers for immune therapy. Furthermore, results showed a correlation between immune therapy effects and HLA matching in preclinical models.

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P03.15 SITE-SPECIFIC IMMUNE EVASION AND SUBSTANTIAL HETEROGENEITY WITHIN ENTITIES PROVIDE EVIDENCE FOR PERSONALIZED IMMUNOTHERAPY

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Background Immune-checkpoint inhibition (CKI) demonstrated remarkable therapeutic efficacy in several kinds of cancer. However, immune escape mechanisms lead to primary or secondary resistance in the majority of patients. Most predictive biomarkers failed, as the primary target of CKI is not the tumor cell itself, but the crosstalk between immune- and cancer cells. We aimed to characterize the immune evasion landscape in primary tumors across different entities.

Materials and Methods Expression of 32 immune-regulatory molecules on lymphocytes was analyzed in peripheral blood and tumor infiltrating lymphocytes (TILs) of 146 primary tumor patients across 10 different entities using flow cytometry. NanoString was applied to determine RNA expression of the respective ligands and 20 genes associated with antigen presentation. Expression of coinhibitory ligands on tumor cells was assessed by immunohistochemistry. To quantify the immune cell infiltration, digital pathology was used and the Immunoscore was generated for each patient.

Results While an increase of regulatory T cells was a common feature across all entities, we found site-specific differences regarding other lymphocyte subsets and expression of immune-regulatory molecules by TILs and tumor cells. Expression of co-inhibitory molecules on tumor infiltrating T cells accumulated especially in advanced stage cancers whereas immune cell infiltration was mainly associated with enhanced antigen presentation. Co-expression of multiple immune-inhibitory ligands was most frequent in colorectal, lung and ovarian carcinoma. Genes related to antigen presentation were frequently dysregulated in seminoma, liver and lung cancer.

Conclusions Immune evasion is a common feature of cancer and frequently detected co-occurrence of multiple mechanisms probably contributes to resistance against immunotherapy. We describe substantial heterogeneity regarding immune escape mechanisms between patients with the same primary tumor. Individualized immunotherapeutic strategies based on pretherapeutic evaluation of the immune evasion landscape might help to improve response to CKI.

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P03.16 FUNCTIONAL DEFECTS IN B-CELLS OF PATIENTS WITH VON-HIPPEL-LINDAU SYNDROME

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Von-Hippel-Lindau (VHL)-disease is an inherited cancer syndrome characterized by a variety of benign and malignant tumors, which develop upon mutation of the second allele of the VHL-tumor suppressor gene. The VHL-protein (pVHL) regulates hypoxia-induced transcription factors (Hif) and by this plays a central role for metabolic cellular adaptations to hypoxic conditions. VHL/Hif regulation plays a well-established role in the development and function of immune cells and already VHL-haploinsufficiency can alter gene expression patterns. In contrast, little is known about primary immune cell functions in VHL-patients. In this study, we analyzed the functional capacity of CD40-stimulated B-cells to act as antigen-presenting cells. We confirmed mono-allelic VHL-gene mutations in B-cells from thirteen VHL-patients and found that their response to CD40-stimulation was significantly reduced. On a functional level this translated to an impaired ability to act as antigen presenting cells leading to impaired T-cell responses in vitro. Taken together, we demonstrate that VHL-haploinsufficiency deregulates B-cell functions following CD40-activation as a new aspect of VHL-syndrome. (The study was registered in the German Clinical Trial Registry (www.drks.de); ID: DRKS00012413).

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P03.17 UPA-PAI-1 HETEROMERS PROMOTE ADVANCED STAGES OF BREAST CANCER BY ATTRACTING PRO-TUMORIGENIC NEUTROPHILS

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Background High tumor levels of urokinase-type plasminogen activator (uPA)-plasminogen activator inhibitor-1 (PAI-1) heteromers independently predict poor survival in early breast cancer. The pathogenetic role of this protein complex, however, remains largely obscure.

Materials and Methods Neutrophil trafficking was analyzed in orthotopic (multi-channel flow cytometry) and heterotopic (ear; multi-channel *in vivo* microscopy) mouse models of 4T1 breast cancer, in a mouse peritonitis assay (multi-channel flow cytometry), as well as in the mouse cremaster muscle (multi-channel *in vivo* microscopy). Cytokine expression in tumors was determined by multiplex ELISA. Phenotypic and functional properties of primary mouse neutrophils, microvascular endothelial cells (cell line bEnd.3), macrophages (cell line RAW 264.7), and breast cancer cells (cell line 4T1) were characterized in different *in vitro* assays. uPA/PAI-1 expression and neutrophil infiltration in human breast cancer samples were assessed by RNA sequencing, immunhistochemistry, and ELISA.

Results Here, we demonstrate that uPA-PAI-1 heteromerization multiplies the potential of the single proteins to attract pro-tumorigenic neutrophils. To this end, tumor-released uPA-PAI-1 utilizes very low density lipoprotein receptor and ERK mitogen-activated protein kinases to initiate a pro-inflammatory program in peritumoral macrophages. This promotes neutrophil trafficking to cancerous lesions and primes these immune cells towards a pro-tumorigenic phenotype, thus supporting tumor growth and metastasis. Blockade of uPA-PAI-1 heteromerization by a novel inhibitor effectively interfered with these events and prevented tumor progression.

Conclusions Our findings identify an already therapeutically targetable interplay between hemostasis and innate immunity that drives advanced stages of breast cancer. As a personalized immunotherapeutic strategy, blockade of uPA-PAI-1 heteromerization might be particularly beneficial for patients with highly aggressive uPA-PAI-1^{high}tumors.

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P03.18 ABSTRACT WITHDRAWN

P03.19 EVALUATION OF IMMUNOGENICITY DIFFERENCES IN LLC1 AND GL261 TUMOR MODELS FOR EFFECTIVE CHEMO-IMMUNOTHERAPY TREATMENT

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Background Tumor immunogenicity is a critical factor responsible for the limited success of cancer immunotherapy and determine the need for personalized treatment. Correct evaluation of effectiveness of cancer treatments and their combination is inseparable from the proper selection of the experimental tumor model. The lack of knowledge about the immunogenicity of animal tumor models makes it difficult to evaluate the efficacy of cancer immunotherapy and becomes

the reason why the results of experimental studies are not suitable for biomedical research. The goal of our work was to evaluate the immunogenic properties of two murine cancer models - Lewis lung carcinoma LLC1 and glioma GL261 and to select two immunologically different tumor models for further chemo-immunotherapy research.

Materials and Methods Firstly, the immunological properties of GL261 and LLC1 cells were assessed in vitro. For this reason, expression of MHC I, PD-L1 and CD44 on LLC1 and GL261 cells surface was evaluated. Then the ability of GL261 and LLC1 lysates to activate immature murine dendritic cells (DCs) was estimated. Murine DCs were generated from bone marrow cells by cultivating them with GM-CSF for 6 days[1] and then maturing them for 24 hours with LLC1 and GL261 lysate supplemented with E. coli lipopolysaccharide. Activation status of DCs was assessed by the expression of surface markers CD11c, MHC II, CD80, CD86, CD40 and CCR7. Later C57BL/6 mice were inoculated s.c. into the left side of the back with GL261 or LLC1 cells. Tumor develpoment was monitored every 2–3 days and then tumors reached a size of ~1.5 cm3 mice were sacrificed. Tumors were collected for evaluation of immune cell infiltration and predominant cytokine profile. Also inactivated GL261 and LLC1 cells were inoculated prophylactically before tumor inoculation and their ability to induce antitumor immune memory was investigated.

Results Our study revealed different immunogenic properties of LLC1 and GL261 cells. LLC1 tumors developed significantly faster than GL261 tumors. Infiltration of immune cells, especially CD8+ lymphocytes and NK cells, was more prominent in GL261 than in LLC1 tumors. Also MHC I and PD-L1 expression was significantly higher on GL261 cells. They also showed better ability to induce antitumor immune memory and to activate murine dendritic cells. Cytokine profile analysis further confirmed immunological differences between LLC1 and GL261 cells.

Conclusions LLC1 and GL261 tumors possess different immunogenic properties - GL261 tumor reflects immunogenic tumor model while LLC1 tumor - nonimmunogenic model. These results confirm us the idea that the immune subtype of tumour should be taken into account when evaluating the results of variuos combinations of chemo-immunotherapies.

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P03.20 A MURINE, MYC-DRIVEN LYMPHOMA MODEL EXPRESSING HUMAN CD22 ENABLES TESTING OF TARGETED THERAPIES AND THEIR EFFECTS ON TUMOR IMMUNE MICROENVIRONMENT

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Background The tumor microenvironment (TME) is composed of various cell types which closely interact via cell cell contacts and cytokines leading to tumor promotion, immune cell inhibition and drug resistance. TME is increasingly recognized for its role in cancer immunotherapies. In B-cell malignancies, myeloid cells play a central role in supporting tumor growth and immune suppression (Roussel *et al*., 2017, Cancer Immunol Immunother). Despite the importance of a syngeneic TME, preclinical studies with novel drugs have mainly been performed in models lacking a functional immune system. Therefore, we developed an immune competent murine lymphoma model transgenic to human CD22 to study effects of targeted therapies on TME.

Materials and Methods A chimeric CD22 consisting of human extracellular and murine intracellular CD22 (h/mCD22) was introduced in BL6 mice ($BL6^{h/mCD22}$). Crossbreeding with $BL6^{\lambda\text{-}myc}$ lead to spontaneous development of murine lymphoma that were serially transplanted. Tumor infiltration and TME was characterized by flow cytometry. Mice were treated with Moxetumomab pasudotox, a CD22 targeted immunotoxin and Doxorubicin.

Results Spontaneously developed tumors in lymphoid organs from $BL6^{h/mCD22\ x\ \lambda\text{-}myc}$ consist of a monomorphic population of $h/mCD22^+$ murine B cells. Three primary lymphoma subclones were isolated from distinct mice and serially transplanted in syngeneic mice. Stable tumor growth was established after subcutaneous (sc) and intravenous (iv) injection. However, TME of sc tumors was infiltrated by less than 1% immune cells, while myc-driven lymphoma in humans usually show substantial immune infiltration. In contrast to sc tumors, systemically growing lymphoma in murine bone marrow (BM) are infiltrated by 30% myeloid cells and 1% T-cells and in murine spleen by 10% and 30%, respectively. Myeloid cells found in these tumors were shown to suppress T cell proliferation *in vitro*. To test functionality of the h/mCD22 transgene, lymphoma-bearing mice were treated with Moxetumomab, which reduced BM lymphoma infiltration by 20 to 100-fold and infiltration in spleen by 5 to 20-fold in the three lymphoma models. Effects of treatment on TME were analyzed after treatment with Doxorubicin which is known to activate myeloid cells *in vivo*. Compared to untreated controls, Doxorubicin increased $CD11b^+$ cells in spleen by 1.5-fold. Among these cells, $Ly6G^+$ granulocytic cells increased most substantially.

Conclusions We established primary, myc-driven $h/mCD22^+$ B-cell lymphoma which stably engraft in syngeneic mice with a TME mimicking myc-driven lymphoma in men. The model responds well to CD22-targeted therapy and Doxorubicin induces expected immunologic changes. Therefore, our unique model provides a platform to test CD22-targeting treatment strategies in an immune competent background.

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P03.21 PROJECTING T CELLS INTO A REFERENCE TRANSCRIPTOMIC ATLAS TO INTERPRET ANTITUMOR IMMUNE RESPONSES

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Background Single-cell transcriptomics is a transformative technology to explore heterogeneous cell populations such as T cells, one of our most potent weapons against cancer and viral

infections. Recent advances in this technology and the computational tools developed in their wake provide unique opportunities to build reference cell atlases that can be used to interpret new single-cell RNA-sequencing (scRNA-seq) data and systematically compare data sets derived from different models or therapeutic conditions.

Materials and Methods We have developed ProjecTILs (https://github.com/carmonalab/ProjecTILs), a novel computational method to project new data sets into a reference map of T cells, enabling their direct comparison in a stable, annotated system of coordinates. ProjecTILs enables the classification of query cells into curated, discrete states, but also over a continuous space of intermediate states. We illustrate the projection of several data sets from recent publications over two cross-study murine T cell reference atlases: the first describing tumor-infiltrating T lymphocytes (TILs), the second characterizing acute and chronic viral infection.

Results ProjecTILs accurately predicted the effects of multiple perturbations, including the ablation of genes controlling T cell differentiation, such as *Tox*, *Ptpn2*, *miR-155* and *Regnase-1*, and identified novel gene programs that were altered in these cells (such as a *Lag3-Klrc1* inhibitory module), revealing mechanisms of action behind these immunotherapeutic targets and opening new opportunities for the identification of novel targets. By comparing multiple samples over the same reference map, and across alternative embeddings, our method allows exploring the effect of cellular perturbations (e.g. as the result of therapy or genetic engineering) in terms of transcriptional states and altered genetic programs.

Conclusions The proposed computational method will likely contribute to reveal the mechanisms of action of experimental immunotherapies and guide novel therapeutic interventions in cancer and beyond.

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P03.22 REPOLARIZATION OF TUMOR-ASSOCIATED MACROPHAGES FOR IMMUNOTHERAPY OF TUMORS WITH DIVERSE MAJOR HISTOCOMPATIBILITY COMPLEX CLASS I EXPRESSION

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Background Depletion of tumor-associated macrophages (TAMs), which are regarded as M2, pro-tumor cells, is one of the strategies for cancer treatment. However, repolarization of TAMs to the M1 anti-tumor phenotype could constitute an immunotherapeutic alternative for tumors with defective major histocompatibility complex class I (MHC-I), where the anti-tumor effect of cytotoxic $CD8^+$ T cells could be limited.

Materials and Methods In this study, we characterized TAMs from mouse tumor models of human papillomavirus 16-associated tumors, characterized by either reversibly (TC-1/A9) or irreversibly (TC-1/dB2m) downregulated MHC-I expression. Tumors were treated with DNA immunization against the papillomaviral E7 oncoprotein combined with intraperitoneal injection of the synthetic oligodeoxynucleotide ODN1826, a Toll-like receptor 9 agonist. TAMs were characterized *ex vivo* by flow cytometry. *In vitro*, $F4/80^+$ TAMs from naïve tumors were stimulated to M1 or M2 phenotype and co-cultures with TC-1/A9 or TC-1/dB2m cells were established. The cytotoxic effect of polarized TAMs was investigated, and the role of nitric oxide (NO) and tumor necrosis factor (TNF)-α was examined. Finally, interleukin (IL)-10, IL-12 and TNF-α concentrations were determined by ELISA in the culture media from polarized TAMs.

Results We demonstrated that TAMs infiltrated both tumor types and this effect was moderately enhanced after combined immunotherapy. Increase in MHC-II molecules, broadly regarded as an M1 marker, was observed solely in TAMs from treated TC-1/A9 tumors. In contrast, TAMs from TC-1/dB2m tumors expressed high MHC-II levels, regardless of the treatment. Therefore, the new $CD38^+/Egr2^+$ classification[1] was applied and showed to be a better descriptive parameter for M1/M2 TAMs, respectively, because the number of $Egr2^+$ TAMs decreased in both tumor types after combined immunotherapy. While $CD38^+$ TAMs were significantly increased after treatment of TC-1/A9 tumors, they did not increase substantially in TC-1/dB2m tumors. *In vitro*, co-cultures with tumor cells resulted in increase of NO production by M1 TAMs. However, NO and TNF-α contributed to the cytotoxic effect only in TAMs from TC-1/A9 tumor. Finally, *in vitro* polarized M1 TAMs were able to produce TNF-α and IL-10 but not IL-12.

Conclusions Our results showed different effects of immunostimulation on cytotoxicity of TAMs from tumors with distinct MHC-I expression. While TAMs from TC-1/A9 tumors acquired M1 phenotype and became cytotoxic, TAMs from TC-1/dB2m tumors were more resistant to repolarization. This project was supported by grants GA19–00816S provided by the Czech Science Foundation and LQ1604 provided by the Ministry of Education, Youth and Sports of the Czech Republic.

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P03.23 EVOLUTION OF THE IMMUNE LANDSCAPE WITHIN PARTIALLY CONTROLLED MURINE MELANOMA

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10.1136/jitc-2020-ITOC7.61

Background Regulatory T cell (Treg) depletion with antibodies against CD25 is effective in tumor models but response rates are low in poorly infiltrated B16 melanomas. Combination with a tumor vaccine enhances efficacy, but relapse usually occurs following partial control, similar to what is seen clinically. How resistance develops is unknown.

Materials and Methods C57BL/6 mice were injected subcutaneously with B16 cells. Treatments included a depleting mouse IgG2a αCD25 antibody and/or a genetically modified, granulocyte-macrophage colony-stimulating factor (GM-CSF) secreting whole B16 tumor vaccine (Gvax). Changes in the immune landscape were assessed with high dimensional flow cytometry.

Results Compared to monotherapies, combined Gvax/αCD25 significantly delayed tumour growth and prolonged survival, in association with enhanced infiltration of T cells with an activated phenotype. Approximately 50% of mice achieved partial response with relapse at day 35–45 post tumor injection. To characterize immune evolution prior to relapse, we analysed stable, partially responding tumors and paired draining lymph nodes (DLNs). Over time, activated PD-1 +ICOS+TCF7- T cells with an effector memory (CD44 +CD62L-) phenotype fell from 30% to 10% whilst resting, TCF7+ early differentiated cells rose in abundance towards levels seen in untreated tumors. Abundance of Ki67-, resting Tregs also recovered. Similar results were obtained in analysis of DLNs.

Conclusions Combined Treg depletion/whole tumor vaccination therapy is effective in a poorly infiltrated B16 melanoma model. Combined treatment promotes T cell infiltration and activation. In mice achieving a partial response, treatment effects on the immune landscape were observed to decay over time suggesting a return to immune equilibrium. Further studies to explore the mechanistic basis of this observation are underway.

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P03.24 CALRETICULIN EXPOSURE ON MALIGNANT BLASTS CORRELATES WITH IMPROVED NK CELL-MEDIATED CYTOTOXICITY IN AML PATIENTS

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In some settings, cancer cells responding to treatment undergo an immunogenic form of cell death that is associated with the abundant emission of danger signals in the form of damage-associated molecular patterns. Accumulating preclinical and clinical evidence indicates that danger signals play a crucial role in the (re-)activation of antitumor immune responses in vivo, thus having a major impact on patient prognosis. We have previously demonstrated that the presence of calreticulin on the surface of malignant blasts is a positive prognostic biomarker for patients with acute myeloid leukemia (AML). Calreticulin exposure not only correlated with enhanced T-cell-dependent antitumor immunity in this setting but also affected the number of circulating natural killer (NK) cells upon restoration of normal hematopoiesis. Here, we report that calreticulin exposure on malignant blasts is associated with enhanced NK cell cytotoxic and secretory functions, both in AML patients and in vivo in mice. The ability of calreticulin to stimulate NK-cells relies on CD11c+CD14high cells that, upon exposure to CRT, express higher levels of IL-15Rα, maturation markers (CD86 and HLA- DR) and CCR7. CRT exposure on malignant blasts also correlates with the upregulation of genes coding for type I interferon. This suggests that CD11c+CD14high cells have increased capacity to migrate to secondary lymphoid organs, where can efficiently deliver stimulatory signals (IL-15Rα/IL- 15) to NK cells. These findings delineate a multipronged, clinically relevant mechanism whereby surface-exposed calreticulin favors NK-cell activation in AML patients.

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P03.25 NEUTRALIZING EXTRACELLULAR CHP-1 IMPAIRS TUMOR GROWTH AND METASTASIS FORMATION

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Background Found in the extracellular compartment, Heat Shock Proteins (HSPs) are actively secreted proteins that modulate the tumor behavior. Extracellular HSPs play a unique role as extracellular chaperones and receptors-binding molecules, favoring the establishment and maintenance of different cancer hallmarks, including immune modulation and evasion. CHP-1, is a ubiquitously expressed protein with chaperone activity and its high expression correlates with high tumor grade and lymph node positivity in different breast and lung cancer subtypes. In addition, CHP-1 is actively and uncanonically secreted by cancer cells in the tumor microenvironment (TME).

Materials and Methods Sera cancer patients were analyzed for the presence of CHP-1. To assess the role of extracellular CHP-1 (eCHP-1) in the TME, in vitro experiments on different cell populations have been performed. To dissect the molecular mechanisms, through which eCHP-1 induces cancer progression, have been analyzed specific signaling pathways in cancer and immune cells. Immune cell composition in presence of eCHP-1 in tumors has been identified using flow-cytometry. The characterization of eCHP-1 inhibition as therapeutic approach has been conducted in breast and colon cancer pre-clinical models.

Results eCHP-1 activates an autocrine signaling through TLR2, TLR4 and LRP1, promoting tumor progression and metastasis formation in different pre-clinical models. Moreover, eCHP-1 can modulate the immune composition of the TME, making interesting the analysis of its inhibition in cancer immunotherapy.

Conclusions eCHP-1 represents a easy accessible protein for diagnosis and targeting in very aggressive canncers.
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P03.26 IMMUNOPROFILING OF ORAL AND OROPHARYNGEAL TUMORS OF DIFFERENT ETIOLOGY

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Background Head and neck carcinomas (HNC) are the world's sixth most common cancer. Most of HNCs are associated with tobacco and other environmental factors but a growing part of oropharyngeal tumors are caused by persistent infection of human papillomavirus (HPV). Patients with HPV positive cancers have a better prognosis with fewer recurrences. This may be caused by different anti-tumor immune response and immune profile of patients. Multispectral fluorescent immunohistochemistry (fIHC) is a powerful tool for a detailed analysis of the tumor microenvironment. This method allows to access the phenotype and calculate cells in tumor parenchyma and stroma of the tumor since in comparison to flow cytometry, an architecture of the tissue remains preserved. fIHC is uniquely suited to study interaction of immune and cancer cells in situ.
Materials and Methods Number of 97 formalin fixed paraffine-embedded slides of the human HNC tissue with known etiology were examined using 4 different panels of 5 antibodies each. These panels include antibodies suitable for phenotyping of immune cells (CD3e, CD4, CD8, FOXP3) or their functional description (PD1, CTLA4, ICOS, CCR4). Additionally, antibodies against Ki67, VEGF and cell cytokeratin were used. Slides were stained using Opal™ 7-Color Fluorescent IHC Kit (Akoya Biosciences). The quantity of immune cells was evaluated in stroma and tumor compartment using InForm™ 2.4.6. software (Akoya Biosciences). For all patients the demographic and clinical data were available and these patients were followed for up to 18 years.
Results Our results have shown significantly higher abundance of Th and Tc in both compartments of HPV+ samples. Besides HPV etiology Th and Tc in the tumor microenvironment predict independently better survival of patients. We did not observed difference in number of Tregs (characterized as a CD3+CD4+FOXP3+ cells) in tumors of different etiology, but we detected higher number of ICOS+Tregs in stroma of HPV- tumors. We also quantified the subpopulations of Th and Tc cells expressing regulatory receptors PD1 and CTLA4. PD1 showed significantly higher expression on Th and Tc both in tumor and stroma of HPV+ tumors, but CTLA4 expression was significantly higher only on Th located in stroma of HPV- tumors. Moreover, we detected significantly higher VEGF expression in both compartments and higher proliferating activity of tumor cells in HPV- tumors.
Conclusions Detailed analyses of the tumor infiltrating lymphocytes allows for selection of prognostic markers in HNC of different etiology. Our results may also help to understand the better prognosis of HPV+ patients. More detailed survival analyses with inclusion of other clinical and demographic data will be presented.
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P03.27 ROLE OF NOX2 FOR HYPOXIA-INDUCED CHEMORESISTANCE IN ACUTE MYELOID LEUKEMIA

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Background Relapse of acute myeloid leukemia (AML) may arise from residual chemoresistant leukemic cells. A hypoxic tumor microenvironment, such as the bone marrow, is known to enhance chemoresistance in various forms of cancer, including AML. Hypoxia inducible factor 1 alpha (HIF-1α) is an important mediator of cellular adaptation to hypoxia. HIF-1α is a constitutively expressed transcription factor that is rapidly degraded under normoxic conditions after hydroxylation by oxygen sensors, such as the HIF prolyl hydroxylases (PHDs). However, under hypoxic conditions the oxygen sensors lose the ability to induce the degradation of HIF-1α resulting in its stabilization and translocation to the nucleolus where it induces the transcription of genes associated with glucose metabolism, angiogenesis, and cell survival. This may result in proliferation of malignant cells, impaired tumor cell differentiation and chemoresistance. Reactive oxygen species (ROS) have been shown to inhibit PHDs and may thereby stabilize HIF-1α, and may thus contribute to chemoresistance. AML cells may generate ROS via the myeloid NADPH oxidase NOX2. We therefore hypothesized that NOX inhibitors would decrease chemoresistance in a hypoxic environment.
Materials and Methods The wild type (WT) AML cell line PLB-985 and its NOX2 knocked out (KO) counterpart were cultured for five days in hypoxia (1% oxygen) or normoxia (21% oxygen) in the presence or absence of the NOX inhibitors histamine dihydrochloride (HDC), diphenyleneiodonium (DPI) and GSK2795039. Thereafter cells were exposed to the chemotherapeutic agent daunorubicin for 48 hours (in hypoxia or normoxia) and cell death was determined using the XTT assay. Stabilization of HIF-1α was measured either by western blot or flow cytometry. Differentiation of cells was quantified by measuring the expression of CD14 and CD11b by flow cytometry.
Results Hypoxia reduced the sensitivity of WT PLB-985 cells to daunorubicin induced cell death ($P < 0.05$, n=4) whereas NOX2 KO cells were equally sensitive to daunorubicin in hypoxia and normoxia ($P > 0.5$, n=4). Furthermore, NOX2 KO AML cells displayed increased sensitivity to daunorubicin induced killing compared with PLB WT cells in a hypoxic environment ($P < 0.05$, n=4). Preliminary results show that pharmacological NOX inhibition using DPI enhanced the sensitivity of WT AML cells to daunorubicin induced killing. These results suggests that functional NOX2 contributes to chemoresistance in a hypoxic environment. As expected, hypoxia stabilized the expression of HIF-1α in AML cells. Preliminary results suggest that HIF-1α expression was reduced in the presence of NOX inhibitors.

Conclusions Genetic deletion or pharmacological inhibition of NOX2 sensitized AML cells to daunorubicin induced killing in hypoxic environments. NOX2 may thus be a target for overcoming chemoresistance in AML cells in the hypoxic bone marrow environment.

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P03.28 STRUCTURAL CHARACTERISTICS IN TUMOR AND LYMPH NODES AS PREDICTORS OF 3-YEAR METASTASIS-FREE SURVIVAL IN SURGICALLY TREATED NSCLC

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Background Surgery is the treatment of choice for early and for some locally advanced non-small cell lung cancer (NSCLC). Ipsilateral hilar and mediastinal lymph nodes are generally removed at the time of tumor resection and assessed for tumor infiltration. However, in particular in the context of immunotherapy, there is now increased awareness about the physiological role of lymph nodes in cancer. It may be possible to assess immune response by examining the cellular composition of locoregional lymph nodes. We aimed to assess structural characteristics in tumor tissue and affected and unaffected lymph nodes in patients with and without 3-year metastasis-free survival.

Materials and methods Internal hospital databases were screened for NSCLC patients fulfilling inclusion criteria. Data on patients age, sex, surgery type, (neo)adjuvant therapy, tumor characteristics and time and location of relapse was extracted. FFPE tissue blocks of primary tumor, affected and unaffected lymph nodes were collected. Hematoxylin and eosin stainings were obtained and tissues were analyzed (e.g. for B-cell proliferation and macrophage infiltration) in collaboration with an experienced pathologist.

Results A total of 754 NSCLC patients were screened for inclusion criteria. Of these, 71 patients remained in remission for at least 3 years after surgery, and 80 patients had local or systemic relapse within 3 years after surgery. Structural characteristics in tumor and lymph node immune populations differed between patients with and without 3-year metastasis-free survival.

Conclusion Structural characteristics differ between patients with and without relapse. Our findings show that structural markers in tumor and lymph nodes should be taken into account when assessing patient prognosis and relapse risk.

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P03.29 CHARACTERIZATION OF TREATMENT-INDUCED ADAPTIVE IMMUNE RESPONSES IN PANCREATIC DUCTAL ADENOCARCINOMA

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Background Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy marked by poor prognosis and profound drug resistance characterized in more than 90% of cases by KRAS mutations. To recapitulate central aspects of PDAC, we employed genetically engineered mouse models presenting $Kras^{G12D}$ pancreas specific expression. Through a high-throughput combination drug screen with trametinib as backbone we identified a high synergism with the multikinase inhibitor nintedanib, preferentially in mesenchymal PDAC, a subtype of this disease characterized by poor prognosis and therapeutic resistance. This combinatorial treatment, that led to the induction of apoptosis in vitro and disease regression in vivo, was accompanied by a strong tumor infiltration of CD8 positive T cells.

Materials and Methods To characterize the treatment-induced adaptive immune cell infiltration in vivo, we performed orthotopic transplantations of KRAS-driven murine PDAC cell lines presenting mesenchymal and epithelial morphology. The derived control and nintedanib + trametinib treated PDAC tumors were analyzed by multi-color immunofluorescence stainings. We compared the findings to high parameter flow cytometry results.

Results Confocal microscopy of the immunofluorescence stainings revealed an overall increase of tumor-infiltrating lymphocytes (TIL) in the tumors upon combinatorial treatment with substantial differences in quantity and spatial distribution. Tumors derived from a PDAC cell line of epithelial morphology were characterized by few TIL mainly located at the invasive margins of the tumors, while tumors derived from a mesenchymal PDAC cell line showed a strong increase of TIL even in the center of the tumor mass. Furthermore, an increased ratio of CD8 positive cytotoxic T cells to CD4 positive helper T cells as well as a decrease of Foxp3 and CD4 positive regulatory T cells could be observed for tumors derived from the mesenchymal PDAC cell line under combinatorial treatment. To investigate if the observed recruitment of T cells was indispensable for treatment efficacy of the combinatorial therapy, we orthotopically transplanted the mesenchymal PDAC cell line in immunodeficient CD3-Knockout (CD3ko) mice and applied an analogous combinatorial treatment scheme. In the CD3ko mice, the combinatorial treatment did not lead to an increased survival or tumor regression as observed in immunocompetent mice. However, flow cytometry and immunofluorescence stainings revealed an increase of B cells upon nintedanib + trametinib treatment.

Conclusions Our findings indicate a reduced efficacy of the combinatorial treatment in T cell deficient mice, underlining the importance of T cells in treatment-induced anti-tumor responses and enlarging the understanding of the role of TIL in PDAC.

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P03.30 TUMUR MUTATIONS DRIVE DYSFUNCTIONAL T CELL DIFFERENTIATION IN LUNG CANCER

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Background Effective anti-tumour immunity requires cancer antigen expression, but persistent antigen exposure in chronic viral infections and autoimmunity has a detrimental effect on immune function. This is associated with a decline of early differentiated T cell populations in favour of later differentiated, dysfunctional subsets, resulting in an unfavourable skewing of the immune landscape. It is unknown whether this occurs locally within the antigen rich tumour microenvironment, driving immune failure.

Materials and 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. Clonal relationship between T cell populations was determined by T cell receptor (TCR) sequencing. We additionally analysed publically available single T cell RNA sequencing data and bulk RNA sequencing data within TCGA.

Results T cell differentiation skewing (T^{DS}) occurred amongst TILs in association with tumour mutational burden (TMB). Surprisingly, this was most evident within the CD4 compartment that had a greater abundance of central memory cells expressing the key transcription factor TCF7. Amongst CD4 cells, loss of a $PD1^{-}CCR7^{+}$ T central memory population was accompanied by gain in abundance of PD1+ populations with exhausted ($CD57^{-}ICOS^{hi}CTLA4^{hi}$) and terminally differentiated effector ($CD57^{+}Eomes^{+}$) features. TCR sequencing revealed early and dysfunctional differentiated populations to be clonally related and CDR3 clustering analysis showed greater similarity of sequences shared vs. non-shared between subsets, consistent with an antigen driven differentiation process. Similar patterns were observed within the CD8 compartment. Identification of these subsets within single T cell RNA sequencing data revealed shared and distinct functional regulators, suggesting the enhanced effector capability of early compared to dysfunctionally differentiated populations. A validated transcriptional signature of T^{DS} generated using TRACERx samples with paired flow cytometry and RNA sequencing data reflected loss of gene expression downstream of TCF7, and predicted worse survival within TRACERx and multiple TCGA cohorts including lung adenocarcinoma (LUAD).

Conclusions Our finding support a model of neoantigen driven T cell differentiation within the tumour microenvironment that drives the depletion of progenitor-like cells and gain in abundance of dysfunctional subsets, resulting in a loss of immune fitness. Our analysis of transcriptomic data elucidates potential regulatory mechanisms and therapeutic targets within the subsets identified.

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P03.31 SKIN DENDRITIC CELLS IN MELANOMA ARE KEY FOR SUCCESSFUL CHECKPOINT BLOCKADE THERAPY

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Background Immunotherapy of cancer by checkpoint blockade has significantly improved the survival of melanoma patients. However, in patients with tumors that are poorly infiltrated by effector T cells the clinical results are not encouraging. Therefore, combination approaches that enhance pre-existing anti-tumor immunity and reset the patients' immunological status are urgently needed. In this study we used the tg(Grm1)EPv melanoma mouse model that reflects a non-immunogenic tumor microenvironment. In this mouse model, spontaneous melanoma development is driven by the ectopic expression of the metabotropic glutamate receptor-1 in melanocytes, which confers to them a hyperproliferative and anti-apoptotic phenotype. The same alteration has been shown to be present in 40% of melanoma patient samples. The aim of our study was to investigate whether enhancing dendritic cell (DC) numbers and function in the tg(Grm1)EPv mouse model could restore responsiveness to checkpoint blockade.

Material and Methods We used multicolor flow cytometry, gene expression analysis by RNA-seq and microarray to

analyze tumors and tumor-draining lymph nodes (tdLN). With various immunological *in vitro* and *in vivo* assays we determined the functional role of DC in tumor immunity.

Results A loss of skin DC has previously been reported for primary melanoma lesions and we here show that melanoma progression in the tg(Grm1)EPv mouse model coincides with a gradual decrease in the skin cDC2 subset and an upregulation of the inhibitory ligands PD-L1 and galectin-9. Monotherapy with anti-PD-L1 could not delay tumor growth, suggesting that this is a good model to study resistance to checkpoint blockade. We hypothesized that by boosting DC numbers and function we would restore responsiveness to checkpoint blockade. By administering a treatment consisting of systemic Flt3L and intratumoral polyI:C/anti-CD40, we were able to rescue the numbers and function of skin cDC2. Analysis of the treated tumors by flow cytometry showed that the DC boost regimen led to an increased tumor infiltration of activated CD4+ and CD8+T cells. An *in vitro* T cell proliferation assay revealed that dermal cDC2 that had migrated to the tdLN, played a crucial role in this process, since these were able to cross-present endogenous gp100 antigen more efficiently than migratory Langerhans cells and dermal cDC1. CD4+ and CD8+T cells recruited in the tumors of the DC boost treated mice, expressed PD-1 and TIM-3. Therefore, combination therapy with checkpoint blockade of these molecules resulted in increased cytotoxic activity within the tumor and eventually delay of tumor growth.

Conclusions Our results demonstrate that skin DC shape the tumor microenvironment upon immunotherapy and thus, therapies that aim to enhance responsiveness to checkpoint blockade may well benefit from a component that boosts the numbers and the function of skin DC.

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P04 Vaccine Therapy

P04.01 DENDRITIC-CELL BASED IMMUNOTHERAPY TARGETING PANCREATIC AND NSCLC CANCER STEM CELLS

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Background The field of cancer immunotherapy is growing at a fast pace, with new developments in this field leading to a change in cancer therapy. Dendritic cells (DCs) are one of the central tools in cellular anti-tumour immunotherapy and the production of clinical grade monocyte-derived DCs (Mo-DCs) is the most frequent approach for antitumor vaccines production. However, there is a large space for improvement of protocols and a clear need for the establishment of clinical standard operating procedures (CSOP). Cancer stem cells (CSCs) are a recently identified small cell population present in the tumour, resistant to radio/chemotherapy and known to be responsible for disease recurrence. Here, we aim to contribute to the standardization of CSOPs and to target and eradicate CSCs by developing a DC-based immunotherapy vaccine for pancreatic and non-small cells lung cancer (NSCLC), comparing DC loading with CSCs vs. classical tumour lysates.

Materials and Methods CSCs from PANC-1 (pancreatic cancer) and A549 (NSCLC) cell lines were isolated and characterized by RT-PCR and flow citometry. CSCs resistance to chemotherapy was also assessed. *In vitro* anti-tumour cytotoxicity assays were performed. We also defined and compared the effect of 4 culture media during human Mo-DCs production. Three Good Manufacturing Practice (GMP) serum-free culture media for clinical use were tested - DendriMACS, AIM-V and X-VIVO 15. RPMI was used as a comparative term given that it is largely used in pre-clinical research. We characterized DC viability, differentiation, maturation, internalization of tumour lysates, cytokines production and autologous T cell stimulatory capacity, as well as metabolomic profiles by Nuclear Magnetic Resonance (NMR) spectroscopy.

Results CSCs from PANC-1 and A549 cell lines were successfully isolated and overexpressed the stem-like markers NANOG, OCT4, SOX2 and CD133, with resistance to gemcitabine. In terms of differentiation, maturation, antigen uptake capacity and metabolic profiles, AIM-V and X-VIVO 15 present similar results. However, the use of X-VIVO 15 shows an enhanced DC production of IL-12. DCs cultured in X-VIVO 15 and AIM-V media are able to induce a superior stimulation of T cells (CTLs and Th1 responses) while DCs cultured in DendriMACS are more prone to induce Treg polarization. Our data show that X-VIVO 15 and AIM-V culture media are preferable to support the differentiation of DCs to be used in immunostimulatory approaches such as in cancer immunotherapy.

Conclusions Overall, our results demonstrate that blood monocytic precursors present considerable plasticity allowing a tailored differentiation of DCs just by changing the nutritive support. This highlights the need of critically defining the culture medium to be used in DC cancer immunotherapy, attaining the desired cell characteristics and consequent robust clinical responses. We are now assessing *in vitro* anti-tumour cytotoxicity to evaluate if DC loading with CSC antigens can be an efficient immunotherapy strategy to target and eliminate this specific and resistant cancer cell population.

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P04.02 A NOVEL CANCER IMMUNOTHERAPY COMBINES RMVA-CD40L WITH TUMOR TARGETING ANTIBODIES

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Background Virus-based vaccines and appropriate costimulation potently enhance antigen-specific T cell immunity against cancer. In the present study, we exploit both innate and adaptive immune responses triggered by a novel recombinant modified vaccinia virus Ankara (rMVA) encoding a Tumor-Associated Antigen (TAA) and the costimulatory CD40L against solid tumors in combination regimes to overcome tumor-induced resistance to immunotherapy.

Material and Methods Subcutaneous murine tumors were induced in C57BL/6 or Balb/c mice using syngeneic tumor cell lines. When tumors were established (60–80 mm^3) mice were intravenously injected with rMVA-CD40L. Tumor growth monitoring and immune cell analysis was performed.

Results Therapeutic treatment with rMVA-CD40L resulted in the control of established tumors in several independent tumor models. This antitumor effect was based on the generation of non-exhausted, systemic tumor-specific cytotoxic $CD8^+$ T cells that was essential for therapeutic efficacy. Strikingly, rMVA-CD40L also induced strong NK cell activation and enhanced cytotoxicity. Moreover, the combination of rMVA-CD40L and tumor targeting antibodies resulted in increased therapeutic antitumor efficacy. This therapeutic combination relied on Fcγ receptor-expressing immune cells as well as on NK cells.

Conclusion We describe a novel and translationally relevant therapeutic synergy between viral vaccination and CD40L costimulation. We show strengthened antitumor immune responses when both rMVA-CD40L-induced innate and adaptive immune mechanisms are exploited by combining immunotherapeutic regimes, such as TAA targeting antibodies. This finding could have a direct positive impact in therapeutic regimens where TAA targeting antibodies could be employed.

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P04.03 IMMUNE MODULATORY VACCINE DIRECTED AGAINST IDO1-EXPRESSING IMMUNE CELLS ELICITS T CELL-MEDIATED ANTI-TUMOR IMMUNITY AND ENHANCES ANTI-PD1 RESPONSES

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Background Indoleamine 2,3-dioxygenase 1 (IDO1) is a tryptophan-catabolizing enzyme that contributes to immunoregulation at many levels, including suppressing effector T cells and inducing/activating regulatory T cells. Thus far, several therapeutic approaches to target IDO1 enzymatic activity have shown promise in preclinical models, however, results from the first major clinical trial were disappointing. The present study seeks to provide preclinical PoC data for the conceptually unique idea of developing an IDO1-targeted vaccine based on our earlier findings that humans exhibit intrinsic T cell reactivity against IDO1 epitopes[1] suggesting the existence of a T cell-mediated, counter-regulatory mechanism directed against cells that express IDO1.

Materials and Methods IDO1-derived peptide vaccines were identified by measurement of vaccine-induced *ex vivo* response (IFNγ ELISpot) and demonstration of anti-tumor responses in CT26 tumor-bearing mice. To understand the vaccine's mode of action, resected tumors were analyzed by immunofluorescence microscopy and flow cytometry.

Results The CT26 colon carcinoma model was selected for these studies based on evidence of high levels of IDO1 expression and responsiveness to IDO1 inhibition reported for these tumors. *In silico*-predicted $H2^d$ MHC class I and II-restricted IDO1 peptide sequences were tested and vaccine candidates were chosen after confirming *ex vivo* response and anti-tumor response in CT26. Therapeutic treatment of established CT26 tumors with MHC class I- and II-directed, IDO1-derived peptide vaccines elicited anti-tumor responses when administered alone, and the effect was further pronounced when combined, suggesting distinct mechanisms of action. In addition, a combination of IDO1 vaccine with anti-PD-1 antibody produced a combinatorial anti-tumor response beyond what was achieved with either agent alone. Consistent with this observation, adoptive transfer of isolated CD8+ T cells from class I and CD4+ T cells from class II peptide-vaccinated responder mice delayed tumor growth in treatment naïve mice. The class II-directed response was completely IDO1-dependent while the class I-directed response included an IDO1-independent component indicative of antigen spread. Examination into the tumors in vaccinated mice indicated that IDO1 vaccine treatment exerts its effect by selective reduction of IDO1 expression in the tumor microenvironment and concomitant expansion of activated $CD4^+$ and $CD8^+$ T cells.

Conclusions As noted in humans, our data demonstrate that IDO1 is immunogenic in mice confirming that this endogenous protein is excluded from normal tolerance mechanisms. The observed immunotherapeutic efficacy of IDO1 peptide vaccines on their own and in combination with anti-PD-1 antibody support the rationale for ongoing clinical development of IDO1 peptide vaccine-based therapy. Future studies include further differentiation of the vaccine platform against

other IDO1-targeting approaches, as well as decoding the underlying mechanism of cooperativity between anti-PD-1 antibody and IDO1 peptide vaccines.

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P04.04 MULTIFUNCTIONAL ANTIBODY CONSTRUCT FOR *IN VIVO* TARGETING OF DENDRITIC CELLS AS A THERAPEUTIC VACCINATION STRATEGY IN AML

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Background Dendritic cells (DCs) are antigen-presenting cells that induce antigen-specific T-cell responses. Therefore, they are used as tools and targets for anti-tumor vaccination. In contrast to T-cell based immunotherapies, that are often limited to surface antigens, DC-based vaccination strategies open up new therapeutic options by utilizing highly abundant intracellular tumor antigens as a target source. Among those, recent interest has been focused on the identification of neoantigens derived from tumor-specific mutations. Especially mutated Nucleophosmin 1 (ΔNPM1) is a considered candidate for targeted therapy in acute myeloid leukemia (AML). We developed a multifunctional antibody construct consisting of a peptide domain including a variable T-cell epitope that is fused to an αCD40 single chain variable fragment (scFv) with agonistic function to target and activate dendritic cells *in vivo*. To potentiate therapeutic efficacy, toll-like receptor (TLR) agonists can be attached as co-stimulatory domains, thereby aiming to enhance cross-presentation of conjugated (neo)antigens to CD8+ T cells.

Materials and Methods Flow cytometry and microscopy-based binding and internalization experiments were performed using monocyte-derived dendritic cells (moDCs). Upregulation of surface markers (CD80, CD83, CD86, HLA-DR) as well as cytokine secretion (IL-6 and IL-12) indicated DC maturation. To validate peptide processing and presentation, moDCs were co-cultured with autologous as well as allogeneic T cells. IFN-γ and TNF-α secretion served as a readout for T-cell activation, peptide-MHC multimer staining for T-cell proliferation.

Results For proof-of-principle experiments, the multispecific antibody derivative was developed by fusing the αCD40 scFv to a cytomegalovirus (CMV)-specific peptide. The αCD40.CMV construct bound CD40 agonistically and showed efficient internalization into early endosomal compartments on immature moDCs. In co-cultures of immature and mature moDCs with autologous or allogeneic T cells, αCD40.CMV induced a significantly increased T-cell activation and proliferation compared to the control. The co-administration of αCD40.CMV with various TLR agonists as vaccine adjuvants resulted in a significant upregulation of DC maturation markers in comparison to αCD40.CMV only. Interestingly, not all adjuvants were able to enhance the T-cell response. To translate this principle to the AML setting, the CMV peptide sequence was replaced with the ΔNPM1-derived and HLA-A*02:01-binding neoantigen CLAVEEVSL. Cross-presentation to CD8+ T cells transduced with a ΔNPM1-specific T-cell receptor was proven by IFN-γ and TNF-α secretion in co-cultures with moDCs that have been pre-incubated with αCD40.ΔNPM1. The optimal vaccine adjuvant has yet to be identified.

Conclusions We successfully demonstrated the development of a multifunctional antibody construct that specifically targets and stimulates DCs by an agonistic αCD40 scFv. It simultaneously delivers a T cell-specific peptide with a vaccine adjuvant to induce an efficient T-cell response. As neoantigens are promising targets and under intense investigaton, the αCD40.ΔNPM1 fusion protein is of high therapeutic interest. Thus, our approach displays a promising DC vaccination option for the treatment of AML.

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P04.05 MODULATING TUMOR MICROENVIRONMENT WITH ARGINASE-1 SPECIFIC T CELLS

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Background Cancer progression is associated with an increased immune suppression at the tumor site. Arginase-1 is an enzyme well-known for its involvement in metabolic immune regulation. At the tumor site, arginase-1 acts by reducing availability of L-arginine to the infiltrating immune cells thus reducing T cell functionality and proliferation. While arginase-1 is expressed by some tumor cells, it has also been shown to be produced by immune inhibitory myeloid cells, such as myeloid derived suppressor cells (MDSCs), tumor associated macrophages (TAMs) and is associated with poor prognosis. Previously, we demonstrated that spontaneous CD4+ and CD8+ T-cell immune responses against arginase-derived, HLA-restricted peptides can be found in both cancer patients and healthy individuals (Martinenaite *et al*, 2018, DOI: 10.1080/2162402X.2017.1404215). These T cells are present in the memory T cell compartment, and that they are activated in arginase-1 inducing conditions,

such as presence of T_H2 cytokines IL-4 or IL-13 *in vitro* (Martinenaite *et al*, 2019, DOI: 10.1038/s41423-019-0231-3 and DOI: 10.1007/s00262-019-02425-6).

Methods and Results In order to explore if arginase-1-specific T cells have a potential role in modulation of immune homeostasis, human arginase-1-specific memory T cells were isolated and expanded for functional characterization. We show that arginase-1-specific T cells specifically recognize arginase-1 expressing cells, such as mRNA transfected autologous dendritic cells (DCs) and B cells as well as M2 polarized macrophages in vitro. In addition, activated arginase-1-specific T cells produce pro-inflammatory cytokines IFNγ and TNFα. Secretion of TH1 cytokines by these T cells suggests potential role as potent immune modulators in the tumor microenvironment, since many arginase-1 expressing myeloid cells are not terminally differentiated and they can be re-polarized to an immunostimulatory, M1-like phenotype. We also observed that targeting of M2-polarized arginase-1 expressing monocytic leukemia cell line THP-1 with arginase-1-specific CD4+ T cells induces upregulation of PD-L1 on the THP-1 cells. Furthermore, we demonstrate that an arginase-1-derive peptide vaccine has a therapeutic effect in syngeneic mouse tumor models (B16 and MC38), both as monotherapy and in combination with anti-PD-1 treatment. The therapeutic effect was associated with increased immune infiltration in the peptide vaccinated mice compared to the control.

Conclusions Our study provides evidence that immune modulatory vaccination targeting arginase-1 is an intriguing way of targeting the immune suppressive microenvironment.

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P04.06 MUCOSAL IMMUNIZATION WITH A CDC1-TARGETED CTA1 ADJUVANT VACCINE CONFERS PROTECTION AGAINST MELANOMA METASTASIS

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Background Specific targeting of anti-cancer vaccines to dendritic cells (DCs) has been shown to mount efficient immune responses against tumor cells. Classical $CD103^+$dendritic cells (also called cDC1) have an inherent ability to cross-present antigens to $CD8^+$ cytotoxic T cells. Here we have explored an anti-tumor vaccine that specifically targets cDC1 cells for protection against and elimination of metastatic melanoma. The vaccine contains the cholera toxin A1 subunit (CTA1) adjuvant and is targeted to cDC1 cells through an anti-CD103 single chain antibody (CD103 scFv).

Material and Methods C57BL/6 mice were injected with wild type or ovalbumin (OVA) expressing B16 melanoma cells either subcutaneously (s.c.) to establish solid tumors, or intravenously (i.v.) to allow the formation of pulmonary metastases. Before or after establishment of tumors, mice were intranasally inoculated with a vaccine composed of a CD103 scFv element fused to the adjuvant CTA1 and the MHC I H2kd-restricted OVA epitope SIINFEKL together with the MHC II H2kd-restricted OVA epitope p323 or just the p323 peptide alone (*i.e.* CTA1-SIINFEKL-p323-CD103 and CTA1-p323-CD103, respectively). Control mice were inoculated with PBS. The growth of solid tumors was carefully monitored and the development of pulmonary metastases was determined 2–3 weeks after tumor cell injection. In addition, antigen-specific T cell immunity following intranasal immunization was evaluated.

Results Targeting MHC I and MHC II tumor cell epitopes to cDC1, via CD103 ScFv, in conjunction with the CTA1 adjuvant elicited strong tumor specific and protective $CD8^+$ T cell responses as well as $CD4^+$ T cell immunity. Immunization with the CTA1-SIINFEKL-p323-CD103 vaccine significantly reduced the growth of established solid B16F1-OVA melanomas ($P<0.001$) and potently prevented metastasis formation ($P<0.01$). Control immunizations with the CTA1-p323-CD103 vaccine tended to reduce metastasis, but tumor-specific $CD8^+$ T cells were required for full therapeutic protection.

Conclusion Targeting tumor specific $CD8^+$ T cell epitopes to cDC1, in the context of a powerful adjuvant such as CTA1, leads to the development of efficient anti-tumor immune responses. Our results point towards the utility of cDC1-targeted vaccines in the treatment of established tumors or as a means to prevent metastasis formation.

Disclosure Information M. Arabpour: None. **S. Paul:** None. **R. Kiffin:** None. **H.G. Wiktorin:** None. **K. Hellstrand:** None. **N. Lycke:** None. **A. Martner:** None.

P04.07 ABSTRACT WITHDRAWN

P04.08 VIRUS LIKE VACCINES: A NOVEL IMMUNOTHERAPY STRATEGY AGAINST THE CANCER-ASSOCIATED ENDOGENOUS RETROVIRUS

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In face of the necessity of broadly acting and highly effective vaccines capable of eliminating/preventing human cancers with insufficient mutated antigens, we introduced the concept of Virus-Like Vaccines (VLVs). This strategy combines a replication-deficient retrovirus encoding virus structural proteins. These proteins assemble into secreted virus-like particles (VLPs) that deliver the target antigen to the immune system rising both humoral and cellular immune responses. Here, we use an adenoviral vector encoding the group specific antigen (Gag) and the glycoprotein of the viral envelope (Env) from endogenous retrovirus (ERV). Since ERV Env is reported to have immunosuppressive properties that support tumor establishment and development, we designed a modified vaccine that includes a mutation on the Env immunosuppressive domain (ISD) that

prevents the vaccine from being immunosuppressive itself. In our studies, we demonstrate that VLVs are able to induce strong, broad, and long-lasting ERV Env specific CD8+ T cell by flow cytometry and antibody responses by ELISA in mice. Furthermore, the modified vaccine is of special interest to future research as it proved to significantly delay mouse tumor growth in a therapeutic setup. Nevertheless, we now need to address the principal host related developmental uncertainties in translating our achievements into the clinical setting. This goal can be accomplished by raising human T cells capable of targeting human cancers *ex vivo*. Furthermore, to support the translation of our work, we tested the ability to rise adaptive responses upon vaccination in non-human primates (NHP) which endogenously express ERVs similar to humans (in collaboration with IPB University, Indonesia). Fellowship granted by Innovation Fund Denmark.

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P04.09 DEVELOPMENT OF A DENDRITIC CELL VACCINE AGAINST HEPATOCELLULAR CARCINOMA USING VSV-NDV

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Background Activated **Dendritic cells (DC)** are the immune system's allrounder: they initiate innate and adaptive immune responses; they induce instant immune reactions as well as immunologic memory. Therefore, there is growing interest in using them as a potential anticancer vaccine.[1] Here we use the beneficial immune-stimulatory properties of the novel oncolytic hybrid virus **VSV-NDV** to create a DC vaccine against hepatocellular carcinoma.[2]

In our therapeutic approach, a sample of the patient's tumor cells is lysed *in vitro* with VSV-NDV (=oncolysate). The patient's DCs are then co-cultured *in vitro* with the oncolysate in order to activate them and load them with tumor antigens. In the end, the stimulated DCs are injected into the patient, where they can lead to a personalized and broad anti-tumor immune response.

Materials and Methods To investigate the potential of the approach in a cell culture system, human monocyte-derived dendritic cells were generated from PBMCs of healthy donors and incubated with VSV-NDV-lysed HepG2 hepatoma cells. Afterwards their state of activation was investigated via flow cytometry and cytokine measurement, whereas their functionality was assessed in co-culture with T-cells. In a murine system, dendritic cells were generated from bone marrow stem cells, incubated with a VSV-NDV-lysed murine HCC clone and investigated as in the human system.

Results Flow cytometry of oncolysate-stimulated DCs showed a significant upregulation of the activation markers CD86, MHC-I, MHC-II and PD-L1 ($p < 0.05$). Moreover, these stimulated DCs released increased amounts of cytokines. Upon co-culture of the DCs with T-cells, an elevated secretion of IFNγ by the T-cells, as well as an upregulation of T-cell activation markers could be shown, demonstrating the functional potential of the oncolysate-stimulated DCs. These results apply to both the human and the murine system.

Conclusions Our *in vitro* data demonstrates that the oncolysate-stimulated human and murine DCs are not only activated, but furthermore have a high functional potential. Further *in vitro*-experiments will be necessary to translate the process to patient-derived samples, whereas murine *in vivo*-experiments will give further insights into the effect of the therapeutic approach.

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P05 Precision Medicine Meets Immunotherapy (Immuno-Monitoring)

P05.01 COMPARATIVE ANALYSIS OF RNA VERSUS DNA AS INPUT MATERIAL FOR IGH REPERTOIRE SEQUENCING PANELS FOR IMMUNO-ONCOLOGY APPLICATIONS AND RARE CLONE DETECTION

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Background Recent progress in tumor immunotherapies have shown the importance of next generation sequencing (NGS) T cell repertoire profiling to characterize T cell immune response to treatment. Understanding the role of the B cell repertoire upon stimulation of the immune system by checkpoint blockade is paramount for immunotherapeutic approaches in treatment of B cell malignancies, as well as understanding B cell function within traditional I/O strategies. The ability to detect low frequency B cell clones enables numerous hematology/oncology research applications, including identification of potential biomarkers and minimal residual disease (MRD) research. Historically, efforts to track the frequency of malignant B cells by IGH chain sequencing have utilized DNA input given potential challenges in accurately quantifying

template copy number from RNA data owing to B cell subtype specific variation in the expression of the B cell receptor. Hypothetically, however, RNA input based monitoring could be advantageous both owing to reduced input requirements and superior ability to detect B call malignancies of plasmablast and plasma cell origin, where the BCR is robustly expressed. Here we compared the ability of RNA and DNA based IGH chain sequencing to detect Burkitt's Lymphoma cell lines and Chronic Lymphocytic Leukemia samples at a frequency of 10^{-6} from peripheral blood.

Materials and Methods Here we present performance for rare clone detection utilizing the Ion Oncomine^{TM} BCR IGH-SR assay and the Ion Oncomine^{TM} BCR IGH-LR assay. These assays use multiplex primers targeting all known IGH germline variable genes in the framework 1 (FR1) or framework 3 (FR3) regions of the B cell receptor using either DNA or RNA as input. To evaluate detection sensitivity of the IGH-SR assay we utilized DNA or RNA from Burkitt's lymphoma cell lines as well as clinical chronic lymphocytic leukemia (CLL) samples controllably added to a background of peripheral blood leukocytes (PBL) by mass ratio to create specimens with a known target B cell frequency. Automated downsampling analysis was used to confirm libraries were sequenced to saturation. Library preparation and analysis was performed in replicate to quantify sensitivity of detection.

Results For each cell line, we prepared and sequenced (1) 30 libraries derived from amplification of 2ug gDNA spiked with 2pg cell line gDNA and (2) 10 libraries derived from amplification of 100ng RNA spiked with 0.1pg cell line total RNA. The Burkitt's lymphoma cell line and CLL samples were detected in 10/30 and 8/30 libraries respectively, consistent with the performance of orthologous DNA-based sequencing approaches. For RNA libraries, the Burkitt's lymphoma and CLL samples were detected in each library (10/10 and 10/10, respectively).

Conclusions Here we demonstrate the ability to detect B cell clones down to 10^{-6} from gDNA and RNA inputs utilizing the Ion Oncomine^{TM} BCR IGH-SR assay. Feasibility for rare clone detection is shown in gDNA or RNA enabling B cell minimal residual disease research, and high sensitivity characterization the B cell role in response to checkpoint blockade within the tumor microenvironment. Importantly, we find that RNA based IGH sequencing may significantly reduce input requirements for rare clone detection, potentially enabling routine detection of clones at 10^{-6} frequency from a single library.

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P05.02 AN INTEGRATED VIRTUAL TISSUE PLATFORM FOR INCORPORATING EXERCISE ONCOLOGY INTO IMMUNOTHERAPY

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We introduce a novel in silico platform for simulating solid tumor growth and anti-tumor immune response. We present the model, test the sensitivity and robustness of its parameters, and calibrate it with pre-clinical and clinical data from exercise oncology experiments which offer a natural biological backdrop for modulation of anti-tumor immune response. We then perform two virtual experiments with the model that demonstrate its usefulness in guiding pre-clinical and clinical studies on immunotherapy. The first virtual experiment probes the intricate dynamics in the tumor microenvironment between the tumor and the infiltrating immune cells. Such dynamics is difficult to probe during a pre-clinical study as it requires significant of redundancy in lab animals and is time and labor intensive. The result is a time series of spatiotemporal observational 'windows' into the tumor microenvironment that can serve as a platform to test several mechanistic hypotheses on the role and dynamics of different immune cells in ant-tumor immune response. The second virtual experiment shows how dosage and frequency of immunotherapy drugs can be optimized based on the aerobic fitness of the patient, so that possible adverse side effects of the treatment can be minimized.

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P06 Cell Therapy in Solid Tumors

P06.01 BISPECIFIC ANTIBODY-DRIVEN SYNTHETIC AGONISTIC RECEPTOR – TRANSDUCED T CELLS MEDIATE SPECIFIC AND CONDITIONAL THERAPY IN MELANOMA CANCER MODELS

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Background Immunotherapeutic approaches, including immune checkpoint blockade and adoptive T cell therapy (ACT) in the form of tumor-infiltrating lymphocytes (TILs), have had marked success in the treatment of melanoma. Despite these successes, many patients are refractory to treatment or relapse with therapy-resistant disease. To overcome these limitations, we propose a controlled ACT approach, where T cells are armed with synthetic agonistic receptors (SARs) that are conditionally activated only in the presence of a target melanoma-associated antigen, and a cross-linking bispecific antibody (BiAb) specific for both (SAR) T cell and tumour cell.

Materials and Methods A SAR composed of an extracellular EGFRvIII, trans- membrane CD28, and intracellular CD28 and CD3z domains was fused via overlap- extension PCR cloning. T cells were retrovirally transduced to stably express our SAR construct. We validated our approach in two murine as well as two human cancer models expressing our melanoma-associated target antigens TYRP (murine) and MCSP (human). We confirmed conditional and specific stimulation and proliferation of our T cells, as well as their tumour-antigen-directed cytotoxicity, *in vitro* and *in vivo*.

Results Crosslinking TYRP-EGFRvIII (murine) and MCSP-EGFRvIII (human) BiAb, monovalently selective for our SAR, induced conditional antigen-dependent activation, proliferation

of SAR-T cells and directed tumour cell lysis with specificity towards two TYRP-expressing murine melanoma and two MCSP-expressing human melanoma cancer models. *In vivo*, anti-tumoural activity was mediated by the co-administration of SAR-T cells and BiAb, in an A375 melanoma xenograft model. Further, overexpression of IDO (a key immunosuppressive enzyme implicated in the suppression of T cell function in the tumor microenvironment) in a melanoma model did not influence the killing kinetics of SAR T cells.

Conclusions Here we apply the SAR x BiAb approach in efforts to deliver specific and conditional activation of synthetic agonistic receptor transduced T cells, and targeted tumour cell lysis. The modularity of our platform is key for a targeting approach in a tumor entity with a high mutational load such as melanoma and is fundamental in our drive towards personalised immunotherapies. Further, the SAR approach has demonstrated resistance to IDO-mediated inhibition in the context of melanoma, an interesting axis that requires further investigation.

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P06.02 ENHANCING CAR T CELL PERSISTENCE AND MEMORY THROUGH MODULATING MITOCHONDRIAL FUNCTION

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10.1136/jitc-2020-ITOC7.81

Background CAR T cell therapy for solid tumours has achieved limited success compared to its application to B cell malignancies. One reason for this failure is the low differentiation rate to memory subsets and low persistence of CAR T cells due to activation-induced cell death (AICD) in lymphoid tissue and the tumour microenvironment. In this study, we have expressed the MCL1 gene within CAR T cells to overcome losses by AICD in adoptively transferred T cells. The MCL1 gene expresses two isoforms; the long isoform localises to the outer membrane of mitochondria and inhibits the CD95 signalling death pathway, while the short isoform localises to the inner membrane of mitochondria to enhance mitochondrial oxidation, phosphorylation and fusion. In addition, we have also utilized a microRNA (miR) 429 to promote memory T cell formation through the suppression of genes such as T-cell-restricted intracellular antigen-1 (TIA-1), T cell activation inhibitor, mitochondrial (TCAIM) and mitochondrial fission factor (MFF).

Materials and Methods Overexpression of MCL1 was confirmed at both mRNA and protein level by real time RT-PCR (qPCR) and western blot. Similarly, overexpression of miR-429 was measured by qPCR and specific binding of miR-429 to the 3′ UTR of target genes was confirmed by luciferase reporter assay. Mitochondrial depolarization and cell viability were assessed by TMRE mitochondrial membrane potential assay (flow-cytometry) and resazurin assay. The effect of MCL1 or miR429 overexpression on HER2-CAR T cells was determined by flow cytometry. Soluble leucine-zipper CD95L (https://www.addgene.org/104349/) was expressed and purified from Expi293 cells.

Results Overexpression of MCL1 in both Jurkat T cells and primary human T cells protected cells against mitochondria depolarization as well as the loss of cell viability in response to CD95L-triggering. Expression of miR429 downregulated TIA1, TCAIM and MFF. A HER2-CAR construct with either MCL1 or miR429 in a lentiviral system was successfully designed and transduced into primary T cells. Mitochondria in transduced T demonstrated enlarged and fusion morphology - a classic feature of memory T cells.

Conclusions Overexpressing MCL1 or miR429 significantly improves mitochondrial function in T cells. This approach will be used to increase persistence of adoptively transferred CAR T cells.

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P06.03 C-C CHEMOKINE RECEPTOR 8 TUMOR-DIRECTED RECRUITMENT ENABLES CAR T CELLS TO REJECT SOLID TUMORS

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10.1136/jitc-2020-ITOC7.82

Background CAR T cell therapy is remarkably successful in patients with hematological malignancies, in some cases inducing durable remissions. However, it remains ineffective in solid tumors, in part due to poor T cell infiltration into the tumor mass. Determinants of successful T cell infiltration to the tumor site remain to be defined. In contrast, tumors actively attract T regulatory (T_{reg}) cells for immune suppression through the C-C chemokine receptor 8 (CCR8) - CCL1 axis. As this axis is functional across cancer entities, we postulated that CCR8 could also be used to target tumor-ablating T cells to the tumor site.

Material and methods Murine and human CCR8 have been cloned in a retroviral expression vector. CCR8 can be expressed in murine and human T cells upon transduction. A chimeric antigen receptor (CAR) targeting the murine epithelial cell adhesion molecule (EpCAM) was used for syngeneic pancreatic tumor models and a CAR targeting human mesothelin was used for a xenograft pancreatic tumor model.Mechanistically, we use flow cytometry and multi-photon intra-vital microscopy to interrogate infiltration of CCR8-transduced CAR T cells.

Results Here we show that genetically engineering CAR T cells to express CCR8 improves their migration into solid tumors and allows rejection of tumors that are otherwise resistant to CAR T cell therapy. We demonstrate the capacity of these enhanced CAR T cells to stunt solid tumor growth and improve survival in both murine syngeneic and human xenograft tumor models.

Conclusion Our results demonstrate the viability of using CCR8 to confer T_{reg}cell trafficking-properties in CAR T cells to enable their effectiveness in solid tumors. This receptor may be combined with other promising strategies to improve the efficacy of cellular approaches.

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P06.04 ENHANCING T CELL FUNCTION FOR CANCER IMMUNOTHERAPY BY MICRORNA MEDIATED KNOCKDOWN OF PRKAR1A

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Background Protein Kinase A (PKA) is a heterotetramer holoenzyme that consists of two regulatory and catalytic subunits. During T cell activation, one of the regulatory subunits (PRKAR1A) localizes to the immune synapse, inhibiting several central proteins in the T-cell signalling cascade and leading to T cell inactivation. Previously, the disruption of localisation of PKA type I R1α (PRKAR1A) to the immune synapse using disruptor peptides has been shown to improve chimeric antigen receptor (CAR) T cell function.[1 2] However, the effect of PRKAR1A knockdown in T cells (including CAR T cells) has not been studied yet. In this study, we have utilized microRNAs (miR); miR96/183 or miR155 to knockdown PRKAR1A and explored the advantages of PRKAR1A knockdown on T cell activation and function.

Materials and Methods MicroRNAs (miR); miR96/183 or miR155 were cloned from human genomic DNA into a sleeping beauty system under a doxycycline inducible promoter (TCE). Overexpression of miRNA and target knockdown was assessed at both transcript level (by real time RT-PCR) and/or protein level (by western blot) respectively while target validation was done by luciferase assay. The fate of PRKAR1A knockdown on Jurkat T cells activated with anti-CD3 and anti-CD28 antibodies were determined by measuring IL-2 production (ELISA) and CD69 surface expression (flow cytometry). The effect of miR96/183 or miR155 overexpression in primary T cells expressing HER2-CAR were also compared.

Results We efficiently overexpressed both miRNAs and downregulated PRKAR1A expression in HEK293 cells at both mRNA and protein level. Luciferase assay confirmed miRNA mediated specific knockdown of PRKAR1A; mutated 3'UTR of PRKAR1A was used as negative control. Overexpression of miRNAs also downregulated PRKAR1A expression in Jurkat cells which resulted in enhanced activation (CD69 expression) and IL-2 production following anti-CD3/CD28 stimulation compared to untransfected controls (with normal PRKAR1A expression). Additionally, miRNA 96/183 and miRNA155 were found to target inhibitory proteins of TCR signalling such as CTLA4, Foxo3 and ptpn2 and resulted in superior T cell function. A third-generation lentiviral system has been optimised to express either miR96/183 or miR155 and HER2-CAR in the same vector and currently we are assessing the effect of PRKAR1A knockdown on primary CAR T cells.

Conclusions Overexpressing miRNA for knockdown of inhibitory proteins could be an efficient way of enhancing T cell function against solid tumours. Additionally, co-expressing CAR and miRNAs using lentiviral system would benefit such approaches for cancer immunotherapy.

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P06.05 IDO1-DELETED CAR T CELLS SHOW IMPROVED THERAPEUTIC EFFICACY IN MURINE PANCREATIC CANCER MODELS

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Background Indoleamine-2,3-dioxygenase 1 (IDO1) is a cytosolic enzyme that catalyzes the rate limiting reaction in the kynurenine pathway. Dendritic cells, macrophages and several tumor entities have been described to express IDO1. In the tumor microenvironment IDO1 promotes tryptophan starvation and accumulation of kynurenines which result in T effector cell proliferation arrest and T regulatory cell induction. Additionally, IDO1 possesses two immunoreceptor tyrosine-based inhibitory motifs (ITIM) that upon phosphorylation can act as docking sites for the recruitment and activation of the tyrosine phosphatases SHP–1 and SHP–2 and ultimately to an activation of the non-canonical NF-KB pathway. Whether IDO1 is expressed in T cells and its potential function is unknown.

Materials and Methods Using IDO1-deleted splenocytes from CD4-Cre $Ido1^{fl/fl}$ mice and WT controls, we evaluated the induction of IDO1 in T cells, as well as the effect of IDO1 in T cell proliferation, differentiation and metabolism. Additionally, we compared *in vitro* and *in vivo* the cytotoxic activity of anti-epithelial cell adhesion molecule (EpCAM) chimeric antigen receptor (CAR) T cells using pancreatic tumor cell lines.

Results IDO1 is inducible in primary mouse T cells upon T cell activation and type I and type II interferon signaling. Interestingly, the use of IDO1 knockout CAR T cells prolongs survival and improves tumor control compared to WT CAR T cell treatment in subcutaneous and orthotopic pancreatic cancer models. In vitro, T cell proliferation, differentiation and cytotoxic function is comparable in WT and IDO1-deleted T cells. RNA sequencing, metabolic and in vivo tracking studies are currently being performed to pin down IDO1-intrinsic effects on CAR T cells.

Conclusions IDO1 is expressed in T cells upon T cell receptor and IFN stimulation and appears to negatively affect tumor control mediated by CAR T cells. Specific IDO1 deletion may improve therapeutic efficacy of CAR T cells in solid tumors, such as pancreatic cancer.

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P06.06 ADOPTIVE CELL THERAPY OF TRIPLE NEGATIVE BREAST CANCER WITH REDIRECTED CYTOKINE-INDUCED KILLER CELLS

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Background Cytokine-Induced Killer (CIK) cells share several functional and phenotypical properties of both T and natural killer (NK) cells, and represent an attractive approach for cell-based immunotherapy as they do not require antigen-specific priming for tumor cell recognition, and can be efficiently and rapidly expanded *in vitro*. We recently reported that CIK cells have a relevant expression of FcγRIIIa (CD16a), which can be exploited in combination with clinical-grade monoclonal antibodies (mAbs) to redirect their lytic activity in an antigen-specific manner. Here, we report the assessment and the efficacy of this combined approach against triple negative breast cancer (TNBC), an aggressive tumor that still requires reliable therapeutic options.

Materials and methods Different primitive and metastatic TNBC cancer mouse models were established in NSG mice, either by implanting patient-derived TNBC samples or MDA-MB-231 cells subcutaneously or orthotopically into the mammary fat pad, or by injecting MDA-MB-231 cells intravenously. The combined treatment consisted in the repeated intratumoral or intravenous injection of CIK cells and cetuximab, while the mAb alone or CIK cells plus Rituximab served as control treatments. Tumor growth and metastasis were monitored by bioluminescence or immunohistochemistry, and survival was recorded.

Results CIK cells plus cetuximab significantly restrained primitive tumor growth in mice, either implanted with TNBC patient-derived tumor xenografts or injected with MDA-MB-231 TNBC cell line. Moreover, in both experimental and spontaneous metastatic models the combined approach almost completely abolished metastasis spreading and dramatically improved survival. The antigen-specific mAb favored tumor and metastasis tissue infiltration by CIK cells, and in particular led to an enrichment of the $CD16a^+$ subset.

Conclusions Data highlight the potentiality of a novel immunotherapy approach where a non-specific cytotoxic cell population can be converted into tumor-specific effectors with clinical-grade antibodies, thus providing not only a therapeutic option for TNBC but also a valid alternative to more complex approaches based on chimeric antigen receptor-engineered cells.

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P06.07 CXCR6 EXPRESSION ENHANCES ACCUMULATION OF ANTI-MESOTHELIN CAR T CELLS AT THE TUMOR SITE AND THEIR THERAPEUTIC EFFICACY IN PANCREATIC CANCER XENOGRAFTS

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Background Chimeric antigen receptor (CAR) T cell therapy is currently approved for the treatment of some hematological malignancies. However, CAR T cells have so far lacked efficacy in the treatment of solid tumors. A major hurdle of CAR T cell therapy is the limited infiltration of CAR T cells into tumor tissue. Chemokine receptors enable immune cells to migrate along a chemokine gradient. Here, we show that overexpression of the C-X-C chemokine receptor 6 (CXCR6) enhances CAR T cell accumulation in C-X-C motif ligand 16 (CXCL16)-positive xenograft pancreatic cancer models, resulting in increased anti-tumor potency of anti-mesothelin CAR T cells.

Materials and Methods Human T cells were retrovirally transduced with an anti-mesothelin CAR and CXCR6. NSG mice were injected subcutaneously with mesothelin-CXCL16-overexpressing tumor cells. Mice were treated once with CAR-, CAR-CXCR6- or mock-transduced T cells when tumors were palpable and tumor size was monitored with a caliper. In a separate tracking experiment, subcutaneous tumors were established as described above and the presence of T cells at the tumor site was determined by FACS analysis within one week after adoptive T cell transfer. For orthotopic xenograft experiments mesothelin-CXCL16-overexpressing tumor cells were directly injected into the pancreas of NSG mice and one-time treatment with CAR-, CAR-CXCR6- or mock T cells was performed 5 days post tumor injection.

Results In a subcutaneous xenograft model of pancreatic cancer CXCR6-expressing CAR T cells displayed improved anti-tumoral potency compared to CAR T cells without CXCR6, resulting in prolonged survival of mice and tumor clearance in 9 out of 10 CAR-CXCR6-treated mice. A tracking experiment confirmed the increased accumulation of CAR-CXCR6 T cells compared to CAR T cells at the subcutaneous tumor site, suggesting increased migratory capacity of CAR-CXCR6-transduced T cells towards CXCL16-expressing tumors as the mode of action. Treatment of orthotopic pancreatic cancer xenografts similarly revealed prolonged survival of CAR-CXCR6-treated animals in comparison to CAR-treated animals, suggesting improved anti-tumor efficacy of CAR-CXCR6-transduced T cells.

Conclusions Forced expression of CXCR6 in anti-mesothelin CAR T cells increased the accumulation of CAR T cells at the CXCL16-positive tumor site, resulting in improved survival of treated mice and in complete tumor rejection in the majority of cases. This data reveals the potential of CXCR6 to direct CAR T cells to the tumor site and this approach may therefore be an attractive strategy to target a major pitfall in the translation of CAR T cell therapy to solid tumors.

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P06.08 IMMUNOMODULATORY BIOMARKERS IN NEOADJUVANT CHEMOTHERAPY OF BREAST CANCER

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Background Neoadjuvant chemotherapy (NAC) with epirubicin/cyclophosphamid followed by docetaxel (E/C->D) is

currently one standard-of-care therapy option in women with early, high-risk or locally advanced breast cancer. While some patients respond excellently to preoperative therapy, in other patients significant tumor shrinkage cannot be achieved. We investigated the impact of NAC on circulating immunomodulatory parameters. We also examined whether changes in these parameters correlate with the response to NAC measured by the Residual Cancer Burden (RCB) score determined after neoadjuvant treatment.

Materials and Methods To detect drug-specific effects, two different NAC regimens in primary breast cancer patients scheduled to pre-operative therapy were compared. 39 patients with conventional anthracycline/taxane sequence (E/C->D, n=39) and 40 patients with reverse sequence (D->E/C) were included. Blood plasma samples were collected at three time points - 'baseline' (before NAC), 'midterm' (after the first six cycles of NAC) and 'surgery' (after NAC before operation). The plasma levels of uPA, uPAR, TIM-3, MCP-1, MCP-2, OPG, IP-10, CD 27, Eotaxin, Tweak, TRAIL, PD-L2, M-CSF and VEGF-A were determined either by using ELISA or a multiplex bead array immunoassay.

Results OPG, CD27, MCP-1, MCP-2, CCl19, Tweak, TRAIL, PD-L2 and M-CSF decreased between baseline and midterm in E/D->D patients. However, the majority of patients treated with the reverse sequence showed no such effect. These drug-induced changes correlated with the RCB score. Non-responders (RCB $\geq$ 1.36) showed a significantly different pattern than responders.

Conclusion These data confirm that NAC affects the immune system in a drug-specific manner. Factors correlating with the RCB-score might represent promising biomarkers to predict the response to therapy.

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P06.09 ANTI-HPSMA CAR ENGINEERED NK-92 CELLS: AN OFF-THE-SHELF CELLULAR THERAPEUTIC FOR TARGETED ELIMINATION OF PROSTATE CANCER CELLS

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Background Adoptive cell therapy of malignant diseases takes advantages of the cellular immune system to recognize and destroy cancer cells. Despite the remarkable success in B cell malignancies after adoptive transfer of CD19 CAR T cells, CAR T cell therapy in solid tumors has shown less encouraging clinical results, above all caused by tumor escape mechanisms.In order to overcome such limitations, NK-92, a permanent and IL-2-dependent cell line with a high cytotoxicity *in vitro*, has been engineered in preclinical models with CAR. In this project, we exploited a CAR directed against the human antigen hPSMA that is overexpressed in prostate tumors. This project aimed at transducing NK-92 cell line to obtain a hPSMA-specific CAR NK-92 cell population, to be thereafter characterized *in vitro* and *in vivo* for antigen-specific functional activity.

Materials and Methods NK-92 cell line was transduced with a lentiviral vector (LV) carrying a CAR anti-hPSMA. The cell population obtained was then sorted and analyzed for degranulation capacity, IFNγ production and lytic activity against $hPSMA^+$ (PC3-hPSMA, LNCaP) or $hPSMA^-$tumor cell lines. *In vivo* therapeutic efficacy of CAR-transduced NK-92 was evaluated initially using Winn-Assay and than in subcutaneous and orthotopic tumor models.

Results CAR-expressing LV efficiently transduced NK-92 cells, which in turn produced cytokines, degranulated and exerted a relevant cytotoxic upon challenge with PSMA+ prostate tumor cells, irrespective of 10 Gy γ-irradiation. In all the *in vivo*, tumor models CAR-transduced NK-92 shown a statistically significant inhibition of tumor growth.

Conclusions Chimeric antigen receptor-engineered NK-92 could offer a valid and cost-effective alternative to primary CAR NK or T cells, in particular in cases, where a suitable donor is not available or the sophisticated infrastructure needed for cell isolation, expansion and genetic modification is missing. This work demonstrates that CAR-engineered NK-92 cells display a high and specific recognition of hPSMA+ PC both *in vitro* as is *in vivo*, and could represent an efficient strategy as a new therapeutic intervention against prostate carcinoma, thus paving the way to an Off-The-Shelf cellular therapeutic for targeted elimination of cancer cells and induction of protective antitumor immunity.

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P06.10 SHORT TERM INHIBITION OF CHECKPOINT PROTEINS INCREASES EX VIVO EXPANSION OF TUMOUR INFILTRATING LYMPHOCYTES IN HIGH GRADE SEROUS OVARIAN CANCER

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Background Ovarian cancer is the most lethal gynaecological malignancy, accounting for approximately 185,000 deaths worldwide in 2018. The majority of patients will experience recurrence of disease. Therefore, there is an urgent need for the development of further therapies to improve patient survival. Tumour infiltrating lymphocyte (TIL) therapy has shown clear efficacy in immunogenic cancers, and TIL can be readily expanded ex vivo from samples of high grade serous ovarian cancer (HGSOC). Key indicators of effective TIL products for infusion are high TIL yield and functionality against autologous tumour. Blockade of checkpoint proteins is effective in increasing TIL yield and functional response from ovarian cancer TIL cultures. However, it is unknown whether blockade of other key checkpoints, including programmed death ligand-1 (PD-1), T cell immunoglobulin mucin-3 (TIM-3) and lymphocyte activation gene-3 (LAG-3) increase TIL yield in ex vivo cultures from HGSOC samples.

Materials and Methods TIL cultures were generated from surgically resected HGSOC tumour samples and were incubated with CD3/CD28 Dynabeads. 3000IU/mL recombinant interleukin-2 (IL-2) was added on alternate days for 7 days before beads were removed. 1000IU/mL IL-2 was added on alternate days for a further 12 days of culture. In cohort 1, 10μg/mL αPD-1, αTIM-3 or αLAG-3 antibodies were added at initiation of TIL cultures only. In cohort 2, 10μg/mL αPD-1, αTIM-3 or αLAG-3 antibodies were added on alternate days

until Day 19. Interferon gamma (IFN-γ) release in response to TIL co-culture with autologous tumour cultures was measured with a human IFN-γ ELISA kit. Data are presented as mean ±SEM.

Results Addition of checkpoint inhibitors at the initiation of HGSOC TIL culture in cohort 1 increased TIL expansion above untreated control in αPD-1 (1.20±0.04 fold, P<0.01, n=9) and αLAG-3 (1.31±0.08 fold, P<0.001, n=9) but not αTIM-3 treated cultures. However, intermittent dosing of HGSOC cultures in cohort 2 with either αPD-1, αTIM-3 or αLAG-3 antibodies did not increase TIL expansion above untreated cultures. In cohort 1, IFN-γ secretion was increased above untreated control in at least one culture treated with a checkpoint inhibitor in 5/7 patients. However, there was no overall fold change in IFN-γ secretion in either αPD-1, αTIM-3 or αLAG-3 treated cultures.

Conclusions This data suggests that initial blockade of checkpoint proteins is effective in increasing the ex vivo expansion of TIL from HGSOC tumours, thus providing a method of improving the efficacy of TIL products in ovarian cancer patients.

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P06.11 IMMUNOTARGETING OF CD98HC FOR ELIMINATION OF RADIORESISTANT HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Background Most patients with head and neck squamous cell carcinomas (HNSCC) are diagnosed during a locally advanced stage and may show therapy resistance. Retrospectively, we showed that low CD98hc mRNA and protein levels are significantly associated with better locoregional tumor control in HNSCC patients.[1,2] Inhibition of CD98hc expression decreased tumor radioresistance suggesting that CD98hc could be a target for HNSCC radiosensitization. One of the strategies for radiosensitization is targeted immunotherapy. However, Chimeric Antigen Receptor (CAR)-equipped T-cell therapy cannot be fully controlled. Therefore, we developed a switchable UniCAR system that is in phase I clinical trial (NCT04230265) [3]. UniCAR T cell activity and specificity are controlled by the presence of target modules (TM) with short half-lives.[3] We aim to define the clinical value of treatment approaches by combining radio(chemo)therapy with CD98hc-targeted immunotherapy.

Materials and Methods We have used previously described radioresistant Cal33 HNSCC cells.[2] These tumor cells were cocultured with UniCAR T cells in the presence or absence of a novel CD98 TM. Specific cell lysis in both *in vitro* 2D and 3D cultures and tumor cell targeting in the experimental mice was assessed.[4]

Results Our data shows that CD98-redirected UniCAR T cells can induce cell lysis of radioresistant HNSCC cells *in vitro* and *in vivo* models. The combination of the UniCAR system with radio(chemo)therapy can be potentially used for the improvement of the treatment efficacy in patients with metastatic radioresistant tumors. The most promising combination of therapeutic approaches will be further tested in xenograft tumor models to evaluate the best performing combination of immunotherapy and radio(chemo)therapy.

Conclusions Overall, it was shown that tumor cells with radioresistant properties can be eradicated via the UniCAR system by targeting CD98hc in an antigen-specific manner.

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P06.12 COMBINATION THERAPY OF CAR-NK-CELLS AND ANTI-PD-1 ANTIBODY RESULTS IN HIGH EFFICACY AGAINST ADVANCED-STAGE GLIOBLASTOMA IN A SYNGENEIC MOUSE MODEL AND INDUCES PROTECTIVE ANTI-TUMOR IMMUNITY *IN VIVO*

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Background Checkpoint inhibitors as well as adoptive cell therapy hold great promise for cancer therapy and encouraging treatment responses have already been demonstrated in different cancer indications. Glioblastoma (GB) is the most common and aggressive primary brain tumor. Standard therapy has very limited efficacy in the majority of patients. Analysis of the GB tumor microenvironment (TME) has shown prominent immunosuppressive features including expression of PD-L1 on tumor cells and increased frequency of FOX-P3 positive regulatory T cells. While the surrounding brain is HER2-negative, GB tumors are frequently HER2-positive, suggesting HER2 as a promising target for adoptive immunotherapy.

Previous results from mouse glioma models showed efficacy of CAR-NK cells (NK-92/5.28.z) targeted against HER2 as monotherapy with relatively small tumors, but not with advanced late-stage tumors.

Materials and Methods The murine glioma cell line GL261 was transfected with HER2. Tumor cells were implanted either subcutaneously or orthotopically into C57BL/6 mice and treated either with HER2-specific NK-92/5.28.z cells alone or in combination with an anti-PD-1 antibody. Effects on tumor growth and survival were determined. Lymphocyte infiltration and immunosuppressive TME were characterized in high-dimensional high-throughput analysis via RNAseq and multiplex IHC.

Results Combined treatment with NK-92/5.28.z cells and anti-PD-1 checkpoint blockade resulted in synergistic effects with tumor regression and long-term survival even of advanced-stage tumor bearing mice. Analysis of TME showed enhanced cytotoxic lymphocyte infiltration and altered profiles of exhaustion markers in tumor and immune cells, leading to an altered TME after combined treatment with NK-92/5.28.z cells and anti-PD-1 antibody.

Conclusions These data demonstrate that efficacy of NK-92/5.28.z cells can be enhanced in combination with checkpoint blockade, resulting in successful treatment of advanced tumors refractory to NK-92/5.28.z monotherapy. Furthermore, the combination therapy induces a cytotoxic rather than immunosuppressive TME, leading to a primed immune system. To address this question in a clinical setting, we are preparing a combination therapy cohort as part of our ongoing phase I clinical study (CAR2BRAIN; NCT03383978).

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P06.13 A NOVEL LOCAL TREATMENT APPROACH? TARGETED IMMUNOTHERAPY OF GLIOBLASTOMA VIA AAV-MEDIATED GENE TRANSFER OF CHECKPOINT INHIBITORS THROUGH LOCALLY ADMINISTERED HER2-AAVS IN COMBINATION WITH CAR-NK CELLS

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Glioblastoma (GB) is the most common primary brain tumor which is characterized by a low immunogenicity of tumor cells and a prevalent immunosuppression in the tumor microenvironment (TME). Since expression of PD-L1 on GB cells has been described, immunotherapy with checkpoint inhibitors (CIs) may be a promising approach for GB treatment. However, systemic administration of CIs bears the risk of autoimmune-like side effects. Delivery of CIs through targeted Adeno-associated viral vectors (AAVs) could overcome this problem. While the brain is HER2(ErbB2)-negative, GB are frequently HER2-positive. Accordingly, intratumoral administration of HER2-specific AAVs encoding CIs may represent a promising approach for GB immunotherapy. This approach will be further combined with local injection of HER2-specific CAR-NK cells (NK-92/5.28.z). The CAR-NK cells already demonstrated efficacy in preclinical GB models and are currently under investigation in the CAR2BRAIN phase I clinical trial. AAVs used in this project harbor a HER2-specific DARPin and encode a murine PD-1 inhibitor (aPD-1). Subcutaneous GL261-HER2 tumors were treated locally with HER2-AAVs either alone or in combination with HER2-specific NK-92/5.28.z cells, and tumor growth and survival were monitored. Subsequently, the efficacy of local application will be compared to systemic AAV administration in subcutaneous and orthotopic intracranial tumors. AAV distribution and specific tumor cell targeting will also be analyzed. Furthermore, future experiments will investigate the influence of AAVs on the TME and the immune cell composition of tumors. Transduction efficacy of HER2-AAVs in murine as well as human glioma cells *in vitro* correlates with the level of HER2 expression. Subsequently, aPD-1 is secreted in a time-dependant manner and binds its target on PD-1-expressing cells. Preliminary results suggest combined therapy with aPD-1-encoding HER2-AAVs and NK-92/5.28.z cells to mediate anti-tumor effects *in vivo*. Comparison of local and systemic administration of HER2-AAVs in subcutaneous and intracranial GL26-HER2 tumors is still subject of ongoing investigation, as well as analysis of tumor cell penetration by AAVs *in vivo*. Local therapy with HER2-AAV in combination with HER2-CAR NK cells is a promising novel strategy for GB immunotherapy with the potential to enhance efficacy and reduce side effects, potentially offering perspectives beyond brain tumor medicine.

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P06.14 CHARACTERIZATION OF TUMOR-INFILTRATING T CELLS BY HIGHLY MULTIPLEXED IMMUNOFLUORESCENCE IMAGING

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Background The adoptive cell transfer (ACT) of tumor-infiltrating T 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 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 represents a daunting challenge for widespread therapeutic application. Alternatively, we hypothesize that TRLs could be identified and enriched 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).

Materials and Methods We screened T cell activation and exhaustion markers, among others, on different tumor tissues 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 on a single section. Moreover, flow cytometry and single-cell RNA sequencing analyses of T cells from tumor digests were performed to complement the characterization of TILs.

Results The MICS results highlighted the complexity of the TME, mainly composed of tumor cells, fibroblasts and endothelial vessels. In some cases, an extensive immune infiltrate consisted of T cells, plasma cells, some B cells and distinct myeloid cells was observed. Particularly, CD8 T cells from different tumor areas exhibited a tissue-resident memory phenotype with the expression of CD69, CD45RO or CD103. Activated/exhausted CD8 T cells were homogenously found across the imaged tumor areas. However, there was a tendency to find them in close proximity to tumor cells, especially for CD8 subsets expressing CD39 and other relevant markers, which may suggest the identification of tumor-reactive CD8 T cell populations. Flow cytometry data revealed the presence of similar T cell phenotypes in the patient´s TILs from tumor digests.

Conclusions This 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 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.

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P06.15 HIGHLY MULTIPLEXED, SINGLE-CELL FUNCTIONAL PROTEOMICS OF CAR-T PRODUCTS ENABLES MORE PREDICTIVE PRODUCT CHARACTERIZATION, CELL MANUFACTURING OPTIMIZATION, AND CELLULAR BIOMARKERS ACROSS PRODUCT TYPES

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Chimeric antigen receptor (CAR) T cell therapy has already paved the way for successful immunotherapies to fight against liquid tumors and is quickly expanding to solid tumors. Nevertheless, the biggest challenges are how to evaluate the quality of CAR-T cells and how to predict their in vivo behaviors once reinfused into a patient. In this report, we review single-cell polyfunctional profiling results obtained from several different sets of pre-infusion CAR-T samples, including CD19 CAR-T products from Novartis and Kite Pharma (Gilead), GoCAR-T cell products targeting Prostate Stem Cell Antigen from Bellicum, bispecific CD19/22 CAR-T cells from the NIH, trimeric APRIL-based CAR-T cells targeting both BCMA and TACI from MGH and CAR-T cells targeting glypican 3 in hepatocellular carcinoma from NIH. In each case, CD4+ and CD8+ CAR-T cells were stimulated and subsequently analyzed at a single-cell level using IsoPlexis' IsoCode proteomic chips. Our single-cell data revealed highly polyfunctional and heterogeneous responses across each cohorts. The polyfunctional strength index (PSI) of the pre-infused CAR-T products is significantly associated with the clinical outcome of the patients after receiving the treatment, as well as post-infusion grade 3 + CRS. The CAR-T cells secreted a wide range of cytokines/chemokines in response to antigen specific stimulation and a significant portion of the CAR-T cells were polyfunctional (2 +cytokines/cell). These results highlight the potential benefits of single-cell proteomics to comprehensively understand how CAR-T products behave in response to antigen-specific stimulation. Analyzing the single-cell polyfunctionality of CAR-T profiles also provides a valuable quality check for optimizing the manufacturing process and a powerful tool for next generation biomarker developments.

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P07 Cell Therapy in Haematologic Diseases

P07.01 CD19 CAR T-CELLS FOR RELAPSED/REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA: REAL-WORLD DATA FROM LMU MUNICH

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Background The anti-CD19 CAR T-cell products Axicabtagene Ciloleucel (Axi-cel) and Tisagenlecleucel have been approved by the EMA for the treatment of patients (pts) with relapse/refractory (r/r) diffuse large B-cell lymphoma (DLBCL) in August 2018. In clinical trials, both cell products induced ongoing complete responses in heavily pretreated patients. However, this activity was associated with significant toxicity. We evaluated the outcomes of DLBCL pts treated with Axi-cel and Tisagenlecleucel at the LMU Munich.

Materials and Methods CAR T cell product characteristics, toxicity and response rates of pts treated at our center between January and October 2019 were retrospectively assessed.

Results As of October 2019, 24 out of 34 r/r DLBCL pts (71%) with confirmed CAR T cell treatment indication were leukapheresed. Four apheresed pts died before CAR T cell therapy due to rapidly progressive disease. So far, 17 DLBCL pts have been treated. Median age was 60 years (range 19–74). ECOG was 0–1 in eleven, and 2–3 in six pts. Eight pts had undergone prior stem cell transplant (6 autologous, 2 allogeneic SCT). 13 pts received bridging chemotherapy between leukapheresis and CAR T cell transfusion. Only 6 (35%) of the 17 transfused pts would have met the inclusion criteria of the pivotal clinical trials (JULIET, ZUMA-1).

CRS occurred in all pts (53% CRS °1, 29% °2 and 18% °3) with a median onset on day 2 (range days 0–7) and a median duration of 4 days (range 1–21). Tocilizumab was administered at least once in all pts. Ten pts (59%) experienced Immune Effector Cell associated Neurotoxicity Syndrome (ICANS, 30% °1, 10% °2, 30% °3, 20% °4 and 10% °5) with a median onset between day 7 and 8 and a median duration of 8 days (range 3–49). Cytopenia was significant following CAR T-cell treatment: all but one pts had neutropenia <500/µl for more than seven days.

Response assessment four weeks after CAR T-cell transfusion was available for 15 pts.

Objective response rate (ORR) at this early follow-up was 67%, with complete remission (CR) in four (27%) and partial remission (PR) in six pts (40%). Interestingly, ORR was higher in the four pts not receiving bridging chemotherapy between leukapheresis and CAR T-cell therapy than in pts in which bridging was applied (100% vs. 55%). Responders had significantly higher LDH levels at apheresis, start of lymphodepletion and CAR T-cell transfusion than non-responders.

Conclusions Since January 2019, the CAR T cell program has been successfully initiated at the LMU Munich, and 17 r/r DLBCL pts have been treated at our center to date. CAR T cells induced responses in heavily pretreated pts with response rates within the expected range. Toxicity was significant but manageable in most pts. Involvement of a multidisciplinary ImmunoTaskforce was a key element for adequate patient care. Preliminary data supports the hypothesis that low tumor dynamics are associated with favorable outcomes of CD19 CAR T cell therapy.

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P07.02 HIGH-AFFINITY TCRS SPECIFIC FOR CANCER TESTIS ANTIGENS AS A THERAPY FOR MULTIPLE MYELOMA AND SOLID TUMORS

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Background Cancer Testis Antigens (CTAs) are highly expressed in multiple different tumor types, but silent in normal tissue, except the testis. This tumor-restricted expression pattern makes them an ideal target for adoptive T-cell therapy. However, the responsiveness in clinical setting may be hampered because high-affinity T cells against self-antigens presented in the context of self-HLA are deleted in the thymus by negative selection. In this study, we aim to identify high-affinity T cell receptors (TCRs) specific for CTAs from the allogeneic-HLA repertoire.

Materials and Methods In this study, HLA class I binding peptides derived from different CTA genes were identified by HLA-peptide elution experiments and subsequent mass spectrometric analysis. From the identified peptides HLA tetramers were generated to isolate peptide specific $CD8^+$ T cells from healthy allogeneic donors. Efficacy and safety of the TCRs was determined by various different stimulation assays. The most potent TCRs were sequenced, analyzed and transduced into peripheral $CD8^+$ and $CD4^+$ T cells to confirm CTA specific cytokine production and cytotoxicity.

Results MAGE and CTAG peptides were eluted from multiple myelomas, EBV-transformed lymphoblastic cells, acute myeloid leukemia and ovarium carcinomas. We selected TCRs recognizing 3 different MAGE-A1 peptides in the context of HLA-A*02:01, HLA-A*03:01 and HLA-B*07:02. Furthermore, we selected TCRs specific for MAGE-A3 in the context of HLA-B*35:01 and HLA-A*01:01; TCRs specific for MAGE-A9 in the context of HLA-A*01:01 and TCRs specific for CTAG1 in the context of HLA-A*02:01. The selected T-cell clones demonstrated efficient recognition of MAGE-A1, MAGE-A3 or CTAG1 positive multiple myeloma and solid tumor cell lines without detectable cross-reactivity.

Conclusions We identified multiple different TCRs from the allogeneic-HLA repertoire specific for CTA genes. These TCRs demonstrate efficient recognition and killing of CTA positive multiple myeloma and solid tumor cell lines and did not show any cross-reactivity. The peptides recognized by the TCRs are presented in different HLA alleles. Since, 71% of the world population contains one of these HLA-alleles, a large percentage suffering from a MAGE or CTAG positive tumor could potentially be treated with the identified TCRs by TCR-gene therapy.

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P08 Combination Therapy

P08.01 LOW-DOSE CHECKPOINT INHIBITORS WITH HYPERTHERMIA AND IL-2 ARE SAFE AND EFFECTIVE IN STAGE IV CANCER WITH UNFAVORABLE IMMUNOLOGICAL PROFILE (MSI^{LOW}, PD-L1 UNDER 1%, TMB^{LOW}) – A SINGLE-INSTITUTION EXPERIENCE FROM 2015 TO 2020

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Background Close to 10 million cancer deaths occurred worldwide in 2017 primarily due to stage IV disease, the management of which is still palliative by intent. Differently from melanoma and non-small cell lung cancer, where the use of ground-breaking immune checkpoint inhibitors (ICI) results in a relatively high efficacy, the response rate in many other stage IV tumors, such as gastrointestinal cancers, breast cancers, sarcomas, and part of genitourinary cancers remains low. In addition, administration of this type of cancer immunotherapy is known for its potentially severe and even fatal side effects due to their severe immune-related adverse events (irAEs).

Materials and Methods Here, we report a retrospective analysis of 129 patients with stage IV cancer who exhausted conventional treatments, who were treated by an low-dose ipilimumab (0.3 mg/kg) plus nivolumab (0.5 mg/kg) blockade in

combination with individually dose adapted interleukin 2 (IL-2) treatment under taurolidine protection and locoregional- and whole body hyperthermia.

Results The overall response (OR) rate of the 129 stage IV patients was 49.6% with an objective response (ORR) of 31.8%. In 15 stage I-III cancer patients, the overall response (OR) rate and objective response (ORR) were 93%, respectively. The entire treatment was performed as outpatient therapy which was mostly associated with a toxicity of grade 1–2 (24.4 and 14.9%, respectively), including nausea, diarrhea, skin rash and pruritus, and elevation of liver transaminases during the first 24 hours. Only 4,76% of the patients developed grade III and 1,79% grade IV irAEs, such as autoimmune hepatitis, thyroid problems, acute kidney injury and/or diabetes mellitus. There were no signs of late adverse events from this treatment with follow-up greater than 5 years post therapy.

Conclusions In comparison to the commonly known rates of response and side effects in ICI, we were able to show relatively high response rates in parallel with low toxicity profile by the aid of immune response modifiers.

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P08.02 BERBERINE-LOADED LIPOSOME FORMULATION ENHANCE THE PHAGOCYTIC ACTIVITY OF LIPOSOMAL IMIQUIMOD TOWARDS COLON CANCER CELL LINES

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Background Colorectal cancer is the third most commonly diagnosed malignant tumor, taking fourth place in terms of cause of cancer deaths worldwide.[1] Unfortunately, the ability of the immune system to distinguish its own from foreign cells is often limited. One of the overexpressed receptors is receptor CD47 - widely distributed glycoprotein on the cell surface of various kind of tumors. It plays a role as 'don't eat me' signal by binding with receptor SIRPα, presents on the cell surface of macrophages.[2] Calreticulin, protein occurring on the surface of tumor cells and phagocytes, acts as protein with pro-phagocytic properties. Several natural bioactive substances are predicted to induce immunogenic cell death by translocation calreticulin on the surface of cancer cells which significantly increases the efficiency of their phagocytosis. Moreover, one of the well-known TLR-7 receptor agonists - imiquimod, is involved in phosphorylation of Bruton's tyrosine kinase leading to the appearance of calreticulin on the surface of macrophages, which increases the efficiency of phagocytosis of tumor cells.[3] Combination therapy composed of berberine and imiquimod can be highlighted as effective immunotherapy for colon cancer. However, such an approach remains very limited. Liposomes can serve as promising carriers for targeting delivery and controlled release of anti-cancer agents.

Material and Methods Liposomes were prepared by the thin-film hydration method followed by extrusion. Human colon cancer cell line (LS180 I SW620) and human monocytic cell line (THP-1) were used for experiments. Calreticulin was detected by using confocal microscopy.

Results The work presented aimed to develop novel liposomal formulations of berberine and imiquimod which were examined for their efficacy in combination against colorectal cancer cell lines. Liposomal formulations of both compounds were successfully prepared using active loading method with different pH generating agents. All loading methods showed desired characteristics in terms of mean liposome size and polydispersity. The encapsulation efficiency was higher than 95% for almost all used formulations. The *in vitro* study proved cytotoxicity of berberine loaded liposomal formulations on tested colon cancer cell lines. The results of the immunofluorescence staining indicated that the both compounds triggered calreticulin on the cell surface (colon cancer or macrophages).

Conclusions The combination of both substances in the liposomal form may generate a synergistic effect on phagocytosis of colon cancer cells.

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P08.03 COMBINING PD-1/PD-L1 BLOCKADE AND RANKL INHIBITORS TO TREAT BREAST CANCERS UNRESPONSIVE TO STANDARD THERAPY

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Background In the past decade, immunotherapy using immune checkpoint inhibitors (especially targeting the PD-1/PD-L1 axis) has been demonstrated as a promising strategy for the treatment of cancers that do not respond to classical chemoradiotherapy. Given that cancer cells have the potential to express many immunosuppressive molecules other than PD-L1, the combination of immune checkpoint inhibitors with other drugs thwarting tumor immunosuppressive microenvironment could represent a promising strategy. Among these immunosuppressive molecules, RANKL, a member of the TNF superfamily, which mainly affects the immune system and bone remodeling, has been shown to be a key factor promoting the progression of breast cancer. In addition, RANKL induces the formation of tolerogenic dendritic cells and Treg cells, which promotes immunotolerance to the tumor.

The aim of this research project is to study the impact of several RANKL inhibitors on triple negative breast cancer and to analyze the efficiency of their association with anti-PD-1/PD-L1 agents.

Materials and Methods We studied RANKL and PD-L1 expression in several murine and human breast cancer cell lines by immunohistochemistry. The secretion of RANKL was analyzed

by ELISA. Inhibitors of RANKL were then tested *in vitro*. We selected several RANKL inhibitors: anti-RANKL antibody, RANK-Fc, Isoliquiritigenin and Gallocatechin gallate. The efficacy of these inhibitors was indirectly evaluated with the murine macrophage RAW264.7 cell line which undergoes, in the presence of RANKL, an osteoclast differentiation (TRAP and Cathepsin K expression). The efficacy of RANKL inhibitors was then evaluated, in this model, by RT-qPCR. Apoptosis and proliferation of the cancer cell lines in the presence of the inhibitors were also analyzed.

Results RANKL/PD-L1 expression profile on specimens from each breast cancer subtypes showed that both immunosuppressive molecules are expressed by all breast cancers with a significantly more intense immunoreactivity for triple negative breast cancers. Most of the cell lines expressed both proteins. We found that RANKL is secreted in their extracellular environment. RANKL inhibitors are efficient and will be tested *in vivo*.

Conclusions Several murine triple negative breast cancer cell lines will be sub-cutaneously injected in mice and the efficacy of both RANKL and PD-L1 inhibitors will be evaluated (separately or in combination). The infiltration of tumor microenvironment by different immune cell populations, the presence of metastasis and the tumor growth will be analyzed.

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P08.04 NEOADJUVANT CHEMORADIOTHERAPY WITH SEQUENTIAL IPILIMUMAB AND NIVOLUMAB IN RECTAL CANCER (CHINOREC): A PROSPECTIVE RANDOMIZED, OPEN-LABEL, MULTICENTER, PHASE II CLINICAL TRIAL

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Background Immune checkpoint inhibitors (ICI), such as ipilimumab (anti-cytotoxic T-lymphocyte-associated protein 4; CTLA-4) or nivolumab (anti-programmed cell death protein 1; PD-1) have been proven to be an effective strategy in solid cancers. Nevertheless, ICI seem not to be effective in microsatellite stable (MSS) tumors, as those potentially lack an immunogenic priming. Radiotherapy is capable to induce an immunogenic cell death (ICD) and subsequently an immunogenic tumor immune microenvironment (TIME). Thus, the pro-inflammatory effect of radiotherapy might restore the susceptibility of MSS tumors to ICI, leading to more pronounced tumor shrinkage, as well as to an effective anti-tumor immune response.

Material and Methods In total 80 patients with a locally advanced rectal cancer (LARC) will be randomly assigned in a 30:50 ratio, to receive either standard of care (SOC) neoadjuvant chemoradiotherapy alone (CRT; 50 Gy in 2 Gy fractions with capecitabine 1650 mg/m^2/d over 25 working days) or concomitant with a single dose of ipilimumab 1 mg/kg on day 7 following sequentially 3 cycles of nivolumab 3 mg/kg Q2W starting on day 14. Patients will undergo surgery within 10 to 12 weeks post CRT. The primary endpoint is safety, tolerability and feasibility assessed by the latest Clavien-Dindo classification of surgical complications and the common terminology criteria of adverse events (CTCAE).

Results ClinicalTrials. gov identifier: NCT04124601. Serial liquid (plasma, serum, peripheral blood mononuclear cells) and tissue biopsies will be taken sequentially before, during and after neoadjuvant therapy. Secondary objectives are radiographic (mrTRG) and pathological (TRG) therapy response. Immune cell infiltrate of resected specimen, as well as genomic, transcriptomic, epigenomic and proteomic pattern of sequential liquid and tissue biopsies will be correlated with therapy response and clinical outcome.

Conclusion This is the first in human study, which uses neoadjuvant CRT in LARC patients with concomitant ipilimumab and nivolumab, applied in a sequential approach. A detailed understanding of therapy induced changes during neoadjuvant CRT with concomitant ICI in a human translation setting will allow the application of radiotherapy as a part of novel immunotherapeutic concept. This is an investigator-initiated trial (IIT), which received a research grant and the study medications from Bristol-Myers Squibb (BMS).

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P09 Young Researchers Session

P09.01 ADOPTIVE CELL THERAPY OF HEMATOLOGICAL MALIGNANCIES USING CYTOKINE-INDUCED KILLER CELLS RETARGETED WITH MONOCLONAL ANTIBODIES

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Background Cytokine-Induced Killer (CIK) cells are a population of effector cells that represents a promising tool for adoptive cell therapy. They are easily expandable *ex-vivo*, safe, and exert cytotoxicity against a broad range of tumor histotypes.[1] 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.[2] 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 can be specifically retargeted against B-cell malignancies by combination with anti-CD20 mAbs, namely Rituximab® (RTX) and Obinutuzumab® (OBI).

Materials and Methods CIK cells were obtained from peripheral blood mononuclear cells of healthy donors, and stimulated *in vitro* with IFN-γ, CD3 mAb 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 B-cell lines, primary samples and patient-derived xenografts (PDX) obtained from B-cell lymphoma patients after written informed consent.

Results The combination with both RTX and OBI significantly increased specific CIK cells lysis against several CD20-expressing lymphoma B cell lines, primary tumors from B-cell lymphoma patients and an established PDX, compared to the combination with a control mAb (cetuximab, CTX). NK-depletion demonstrated that the mAb-mediated cytotoxicity is accountable to the CIK cells fraction within the bulk population since no difference in the lytic activity was detectd in the absence of NK cells. In addition, these results are further supported by *in vivo* preliminary experiments where the treatment with CIK cells in combination with OBI extensively reduced the growth of PDX and increased mice survival, compared to CIK cells or OBI administered alone.

Conclusions Here we proved that CIK cells can be retargeted with clinical-grade mAbs against CD20-expressing lymphomas. These data indicate that the combination of CIK cells with mAbs can represent a novel approach for the treatment of haematological malignancies.

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2. Cappuzzello E, Tosi A, Zanovello P, Sommaggio R, Rosato A. Retargeting cytokine-induced killer cell activity by CD16 engagement with clinical-grade antibodies. *Oncoimmunology* 2016 Aug;**5**(8):e1199311.

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P09.02 MAPPING AND TACKLING TUMOR AND CHEMOTHERAPY-INDUCED IMMUNE SUPPRESSION IN BREAST CANCER SENTINEL LYMPH NODES

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Background Breast cancer (BrC) is the most prevalent cancer in women worldwide. Unfortunately, still limited treatment options are available for the most aggressive subtypes (i.e. hormone receptor [HR] negative). The response to neoadjuvant chemotherapy (NACT) in patients with HR-negative BrC can in part be influenced by an effective anti-tumor immune response. The sentinel lymph node (SLN) is the first site where BrC-specific T cell priming will occur but unfortunately it is also a major target of BrC-induced immune suppression. Lymph-node resident dendritic cells (LNR-DC) were found to be suppressed in metastatic SLN.[1] In addition, this tumor-mediated immune suppression of LNR-DC is related to high-risk triple-negative BrC and may be a negative predictor for prognosis[1]. Preliminary data showed that NACT further reduced the activation status of LNR-DC. The goal of this study is to identify immune-enhancing agents that can counteract the tumor-mediated immune suppression of LNR-DC and promote tumor-specific T cell responses in order to improve therapy outcome in BrC patients upon NACT.

Materials and Methods Phenotypic analyses were performed on immune-cell subsets in human BrC SLN using multi-color flow cytometry. In addition, *ex-vivo* cultures with human BrC SLN-derived cells and *in vivo* mouse experiments were performed to study the therapeutic efficacy of Toll-like receptor (TLR)-ligands (R848 and CpG) and a STING-ligand (STING-L; 2'3'-c-di-AM(PS)$_2$(Rp,Rp)).

Results Higher rates of LNR-DCs, but with an apparently reduced activation state, were found in SLN of NACT-treated patients compared to patients treated with surgery only. A comparative *ex-vivo* study with SLN cultures on the effects of R848, CpG-B and STING-L showed R848 to be superior in terms of LNR-DC activation. In a *Krt14 (K14)-cre;Cdh1F/F;Trp53F/F* (KEP) BrC mouse model, the effects of intratumoral administration of TLR- and STING-L were determined in combination with doxorubicin. STING-L outperformed R848 and CpG-B in terms of controlling primary tumor growth. Of note, in human e*x-vivo* cultures CpG-B proved effective in LNR-DC activation when combined with a STAT3 inhibitor, leading to the boosting of mammaglobin-specific T cell responses, Th1 skewing, and a drop in CpG-induced Treg levels.

Conclusions In summary, intratumoral delivery of TLR- and STING-ligands in combination with NACT might be an interesting therapeutic approach in patients with high-risk HR-negative BrC, leading to SLN potentiation and enhanced antitumor T cell immunity. Future clinical studies should demonstrate the therapeutic benefit of this approach.

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P09.03 *CATHEPSIN S* ALTERATIONS INDUCE A TUMOR-PROMOTING IMMUNE MICROENVIRONMENT IN FOLLICULAR LYMPHOMA

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Background By targeted DNA sequencing of 305 diagnostic follicular lymphoma (FL) biopsies, we identified somatic mutations of *Cathepsin S (CTSS)* in 8% of cases (24/305), mostly clustered at Y132 (19/24) converting Y to D (16/19). Another 13% of FL had *CTSS* amplifications (37/286), associated with higher *CTSS* expression (*P=0.05*). CTSS is a cysteine protease that is highly expressed in endolysosomes of antigen presenting cells and malignant B-cells. CTSS is involved in proteolytical processing of antigenic peptides for presentation on MHC-II to be recognized by antigen specific $CD4^+$ T-cells.[1] CTSS is synthesized as an inactive zymogen, which is converted to its active form by autocatalytic cleavage of the autoinhibitory propeptide (pro-CTSS).

Materials and Methods We used CRISPR/Cas9 to introduce *CTSS* Y132D into Karpas422, a B-cell lymphoma cell line that harbors the FL hallmark translocation t(14;18). We purified pro-CTSS WT and Y132D and assayed the *in vitro* autocatalytic cleavage over time. We then tested the impact of CTSS on $CD4^+$ T-cell activation in co-culture assays, in a previously described *in vivo* model[2] which we slightly modified to reflect FL-like conditions, and in primary patient samples.

Results Single-cell derived Y132D mutant Karpas422 clones showed >3-fold higher ratios of active CTSS to pro-CTSS (N=4, *P=0.0003*). Immunoprecipitated CTSS Y132D had >3-fold higher *in vitro* substrate cleavage activity compared to CTSS wild type (WT) (N=6, *P=0.001*) which was mediated by an accelerated conversion from pro-CTSS to active CTSS (11 minutes for CTSS Y132D *vs* 17 minutes for CTSS WT; N=3, *P*=0.04). Molecular dynamics simulations showed that the Y132D mutation shortens the distances by ~2Å between the catalytic triad of active CTSS (C139, H278, N298) and a stretch of amino acids from the proform (L80, G81, D82, S94), which could facilitate intramolecular cleavage. The higher substrate cleavage activity of CTSS Y132D came along with a high capacity to stimulate antigen specific $CD4^+$ T cell responses *in vitro* and *in vivo*. Additionally, *CTSS* overexpression could phenocopy this high $CD4^+$ T cell activation. Lastly, we aimed to correlate *CTSS* aberrations with clinical outcome in patients who received standard immunochemotherapy (R-CHOP) for advanced FL (N=51 with available *CTSS* mutation and gene expression data). Compared to all other patients (N=34), patients with *CTSS* Y132 mutations or *CTSS* overexpression (N=17) had longer failure free survival (*P*=0.012).

Conclusions Here, we provide biochemical, structural, functional and clinical evidence that aberrant CTSS activity induces a supportive immune microenvironment in FL. We propose that aberrant CTSS activity can elicit a $CD4^+$ T-cell driven tumor-promoting immune response, which could be amplified within the microenvironment and substantially impact the biology and clinical course of the disease. Thus, aberrant CTSS activity is a promising biomarker and therapeutic target in FL and potentially also other tumors.

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P09.04 ONCOLYTIC H5N1 INFLUENZA STRAIN DISPLAYS SUPERIOR THERAPEUTIC PROPERTIES INDEPENDENT OF IMMUNO-STIMULATORY INTERLEUKIN-2 TRANSGENE EXPRESSION

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Background Oncolytic viruses are becoming an integral part of immunological approaches to cancer treatment. Interleukin-2 (IL-2) is known to stimulate cytotoxic T-cells, and might therefore be a reasonable cargo to enhance the therapeutic effect of such viruses. However, IL-2 is also known to promote immunosuppressive regulatory T-cells (T-reg). We investigated the impact of virally expressed IL-2 on induction of regulatory T-cells. We further investigated the effect of virally expressed IL-2 on the therapeutic efficacy of influenza A H1 and H5 subtypes.

Materials and Methods Survival of B16 melanoma xenograft bearing mice upon treatment with various oncolytic influenza viruses was examined. Effect of these viruses on PBMC gathered from 4 young healthy volunteers and murine and human melanoma cell lines was examined utilizing multiple flow cytometry protocols.

Results Viral IL-2 expression did not alter viral growth and was stable up to multiple passages in cell cultures. In human PBMC viral expression of IL-2 did not enhance differentiation

of T-cells into a regulatory phenotype. In a murine B16 xenograft model IL-2 expression significantly enhanced therapeutic effects of an H1 oncolytic influenza virus. Expressed within the background of H5 hemagglutinin, IL-2 did not lead to a significant enhancement of therapeutic efficacy. Interestingly, the empty influenza H5 subtype was significantly more potent in treating B16 xenograft tumors than the H1 subtype, regardless of IL-2 expression. In primary human PBMC models, the virus based on H1 hemagglutinin led to enhanced CD8 T-cell activation compared to H5. This effect was further enhanced by IL-2 expression, although all viruses led to significant activation. Surprisingly, viruses based on H1 hemagglutinin led to increased expression of the immune checkpoint PD-1. The virus based on H5 hemagglutinin did not lead to upregulation of PD-1, indicating a favorable balance between activation and exhaustion. Infection with the H5 based virus led to both enhanced apoptosis and immunogenic calreticulin exposure in human and murine melanoma cell lines compared to H1.

Conclusions IL-2 does not promote T-regs, when expressed in a viral background. H1 viruses induced PD-1 more potently than H5 viruses. The choice of viral entry protein is more relevant for the therapeutic effect of the virus, than the expression of a T-cell stimulating cytokine such as IL-2. Efficacy of oncolytic viral treatment appears to depend more on viral growth than on virally expressed T-cell promoting cargo.

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P09.05 IMMUNOGENICITY INDUCED BY THE ACADEMIC CHIMERIC ANTIGEN RECEPTOR CAR19 (ARI-0001) IN PATIENTS WITH CD19-POSITIVE RELAPSED/REFRACTORY B-CELL MALIGNANCIES RECRUITED INTO THE CART19-BE-01 CLINICAL TRIAL

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Background Chimeric Antigen Receptor (CAR)-T cells directed against CD19 have induced high rates of response in patients with relapsed/refractory (R/R) B-cell malignancies. Two CD19-targeting constructs have been approved by the FDA and EMA (Yescarta ®, Kymriah ®) for B lymphoblastic leukemia (B-ALL) and aggressive lymphoma. Despite deep remissions, there are still major challenges and disparate data are reported about the immunogenicity induced by CART-cell therapy. On May/2017, the Spanish Agency of Medicines approved our first clinical trial (clinicaltrials.gov NCT03144583) with a fully academic CART-19.

Materials and Methods Eligibility criteria included R/R B-ALL (adult and pediatric), non-Hodgkin's lymphoma (NHL) and chronic lymphocytic leukemia(CLL) who failed standard therapy. The primary objective of the study was safety and secondary objectives were response rate and its duration. The humoral anti-CART response was assessed by a (cell-based) fluorescence assay to detect human anti-murine antibodies (HAMA) in patients sera. Assessment was conducted at different time points: 1) at baseline (pre-dose), 2) on day 14 after the administration of ARI-0001 cells, 3) on day 28, 4) on day 100, and 5) every 3 months thereafter.

Results Forty-seven patients (37 adults/10 pediatrics) received ARI-0001 cells. Thirty-eight patients had a diagnosis of R/R B-ALL (28 adults and 10 children); all but 5 had relapsed after allogeneic hematopoietic stem cell transplant (HCT). Seven patients had a diagnosis of NHL, four of them (57%) had relapsed after HCT, and 2 patients had a diagnosis of CLL (2). Median age was 27 years (3–68). After conditioning with fludarabine (90 mg/m2) and cyclophosphamide (900 mg/m2), a total dose of 0.5–5 $x10^6$ ARI-0001 cells/kg was infused. Autologous T-cells from peripheral blood were expanded and transduced with a lentivirus to express a CAR with a single-chain variable fragment (scFv) with anti-CD19 specificity, conjugated with the co-stimulatory regions 4-1BB and CD3z. The scFv was originated from a mouse monoclonal antibody A3B1. Twenty-five per cent of the patients tested positive for the presence of anti-CAR antibodies, all of them post-dose, in contrast to previous data reported on Kymriah® with a significant presence of pre-dose anti-murine CAR19 antibody. Of these 12 patients, 8 patients presented with a weak, and 4 patients with a strong presence of HAMA. The last 4 patients had lost the effectiveness of the CART- therapy at that time point, reflected by simultaneous B-cell recovery in the periphery. Moreover, three of them received a second dose of CART-19, which did not revert the relapse.

Conclusions To conclude, these data suggest the importance of the immunogenicity induced by CART-cell therapies. Immune monitoring should include the assessment of humoral response, especially before considering a second dose after relapse.

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P09.06 'AN ENHANCED CRISPR TOOL FOR TREATING CHRONIC MYELOGENOUS LEUKEMIA'

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Background Chronic myeloid leukemia (CML) is a myeloproliferative neoplastic disease, occurring in 1 to 2 cases per 100.000 adults, which accounts this type of cancer for approximately 15% of newly diagnosed leukemia in adult patients. The diagnosis is based upon the genetic translocation between the t(9;22)(q34;q11.2), resulting in formation

of Philadelphia fusion chromosome, coding for BCR-ABL1 oncoprotein. The life-long treatment relies on using tyrosine kinase inhibitors (TKIs). In some cases, patients develop point mutations, leading to resistance to TKIs treatment, nearly in 2%. Allogeneic stem cell transplantation is the possible solution for these individuals in late stages of CML with success cure rate only approximately at 40%.[1] Based on this funding new solutions for treating cancer with genetic etiology are considered. CRISPR/Cas system, composed of guide RNA, targeting endonuclease Cas9 to specific target genomic region has been used before to mediat breakage of Philadelphia chromosome at the site of oncogenic translocation, although at lower efficiency.[2]

Materials and Methods K562 cells, model for Philadelphia chromosome positive cells, were used. Constructs, expressing BCR-ABL1 targeting gRNA and Cas9, tethered via coiled-coil forming peptides to *E.coli* exonuclease EXOIII, were nucleofected into target cells. T7E1 assay to detect genome modifications was carried out. TUNEL assay, FACS analysis and bioluminescence measurement were used for cell death determination. SCID mice were used for a subcutaneous K562 cancer model.

Results Our strategy was to couple Cas9 to the exonuclease to promote large deletion at the target site. Of the different exonucleases tested, the EXOIII exhibited the best performance in terms of deletion formation. To improve the rate of deletion genetic lesions, we connected Cas9 and EXOIII via coiled-coil forming peptides, bringing the two enzymes into close proximity (CRISPR-EXO). This resulted in an increased deletion formation compared to the standard CRISPR/Cas system. We performed a case study for the use of the CRISPR-EXO system as a potential anti-cancer therapeutic tool. In the case of our new system, we showed significant increase in cell death due to higher genome modification in BCR-ABL1 region. Later, these findings were confirmed also in an animal cancer model, where animals with tumors, electroporated with CRISPR-EXO system showed full survival and drastic reduction in tumor size.

Conclusions CRISPR-EXO upgraded CRISPR system based on tethering Cas9 protein to exonuclease EXOIII by heterodimeric coiled-coil forming peptides, resulted in highly efficient editing of BCR-ABL1 fusion gene, leading to enhanced death of CML cancer cells.

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P09.07 AN IMMUNE MODULATORY VACCINE TARGETING CCL22 PROMOTES ANTI-TUMOR IMMUNITY

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Background CCL22 is a macrophage-derived chemokine that exerts immunosuppressive functions by the recruitment of regulatory T cells (Treg) through the CCL22/CCR4 axis. It has been described to play a key role in the suppression of anti-cancer immunity in different cancer types including ovarian, breast, or pancreatic cancer and is thought to promote the suppression of anti-cancer immunity by Treg recruitment. Recently, we described that CCL22-specific T cells generated from cancer patients can kill CCL22-expressing tumor cells and directly influence the level of CCL22 *in vitro*.[1] In this study, we provide PoC data for a CCL22-targeting vaccine by assessing the immunotherapeutic efficacy of this approach in syngeneic mouse tumor models.

Materials and Methods Peptide vaccines that induce expansion of CCL22-specific T cells were identified by measurement of vaccine-induced *ex vivo* response (IFNγ ELISpot) in BALB/c and C57BL/6 mice. The antitumor efficacy was evaluated in CT26, Pan02 and B16 syngeneic models. To investigate the vaccine's mode of action, the tumor immune infiltration was analyzed through flow cytometry and qPCR.

Results Vaccination with CCL22-specific peptide vaccines induced expansion of primarily CD8+, CCL22-specific T cell responses (assessed by *ex vivo* IFNγ ELISpot). Treatment with CCL22 vaccines reduced tumor growth and increased survival in CT26, Pan02 and B16 tumor models. Assessment of gene expression in the tumors indicated that vaccination leads to a reduction of CCL22 expression in the tumor microenvironment (TME), as well as the expression of other immune-suppressive molecules such as IDO. Furthermore, vaccinated mice harbored an increased CD8+ T cell infiltration with a concomitant increase in M1/M2 ratio within the TME.

Conclusions This study provides evidence that targeting CCL22 expressing cells by vaccination induces immune modulation in the TME, leading to augmentation of anti-tumor responses - thus provides a rationale for a novel immunotherapeutic approach in cancer.

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P09.08 CLINICAL-GRADE MANUFACTURING OF ROR1 CAR T CELLS USING A NOVEL VIRUS-FREE PROTOCOL

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Background Immunotherapy with T cells that were modified by gene-transfer to express a ROR1-specific chimeric antigen receptor (ROR1 CAR-T) has therapeutic potential in ROR1$^+$ malignancies in hematology and oncology. The ROR1 tumor

antigen has a favorable expression profile with absence in vital normal human tissues. In this study, we sought to establish and validate clinical-grade manufacturing of ROR1 CAR-T to enable a Phase I/IIa clinical trial. In particular, we sought to integrate virus-free gene-transfer based on *Sleeping Beauty* transposition into this manufacturing protocol to permit scale-up and export to point-of-care manufacturing, and to reduce turn-around time, complexity and regulatory burden associated with conventional viral gene-transfer (biosafety level 2 to biosafety level 1).

Materials and Methods Buffy coats or leukaphereses were obtained from healthy donors to perform protocol optimization (n=7) and scale-up runs (n=1). $CD4^+$ and $CD8^+$ T cells were isolated separately by magnetic selection and stimulated with CD3/CD28 TransACT® reagent. T cells were transfected with mRNA encoding hyperactive Sleeping Beauty transposase (SB100X) and minicircle DNA (MC) encoding a pT2 transposon comprising the ROR1 CAR and an EGFRt marker gene using the MaxCyte GTx ® electroporation platform. Following transfection, T cells were expanded for 10–13 days in G-REX® bioreactors and then harvested and formulated into the drug product at a 1:1 ratio of CAR-expressing CD4:CD8 T cells. The drug product underwent comprehensive phenotypic, functional and genomic analyses as part of product qualification.

Results The set of protocol optimization runs resulted in a highly robust process. On average, the stable gene-transfer rate at the end of the manufacturing process was 71% in CD4+ (n=5) and 54% in $CD8^+$ T cells (n=7). The average yield of ROR1 CAR-T relative to the number of input T cells was 12.6-fold for $CD4^+$ and 9.4-fold for $CD8^+$ after 12–15 days of expansion, with an average viability of 84% for $CD4^+$ and 82% of $CD8^+$ T cells. The scale-up run was performed with a leukapheresis product from which $52.5 \times 10\hat{}6$ $CD4^+$ and $109 \times 10\hat{}6$ $CD8^+$ T cells were transfected. At the end of the manufacturing process (day 12), there were $844 \times 10\hat{}6$ CAR-expressing $CD4^+$ (~16-fold expansion) and $857 \times 10\hat{}6$ CAR-expressing $CD8^+$ T cells (8-fold expansion). In functional testing, ROR1 CAR-T showed specific recognition and potent elimination of $ROR1^+$ target cells, as well as antigen-dependent cytokine production and productive proliferation in *in vitro* analyses. Experiments to determine the anti-tumor potency of the drug product *in vivo* and detailed genomic analyses are ongoing. Preliminary analyses suggest a favorable genomic insertion profile of the CAR transposon, and a transposon copy number that is well within the range acceptable for clinical use of the drug product.

Conclusions With this novel protocol, we aim to obtain the first manufacturing license for CAR-T in Europe that integrates our optimized approach with SB100X mRNA and transposon MC for CAR gene-transfer on the MaxCyte transfection platform. The quality and yield of the drug product support the design and dose escalation of the proposed clinical trial with ROR1 CAR-T, and will serve as a blueprint for other CAR-T products from our pipeline.

Disclosure Information K. Mestermann: None. **M. Eichler:** None. **M. Machwirth:** None. **K. Kebbel:** None. **U. Köhl:** None. **H. Einsele:** None. **C. Müller:** None. **J. Lehmann:** None. **T. Raskó:** None. **F. Lundberg:** None. **Z. Izsvák:** None. **G. Schmiedeknecht:** None. **M. Hudecek:** None.

P09.09 PD-1 CHECKPOINT BLOCKADE FOR TREATMENT OF MUCORMYCOSIS AND INVASIVE ASPERGILLOSIS IN A STEM CELL TRANSPLANT RECIPIENT

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Background Despite early surgical debridement and application of systemic antifungal drugs, invasive fungal infections by *mucor* spp. are still associated with a very poor prognosis in immunocompromised patients. Due to their lack of immune defense, targeted treatment strategies reversing the hyporesponsiveness of the immune system by immune checkpoints might improve patients' outcome. Until today, a successful recovery of mucormycosis after receiving anti-PD-1 antibody is only described once for a polytrauma patient. Therefore, we here describe the first immunosuppressed patient treated with nivolumab for invasive mucormycosis with aspergillus coinfection.

Materials and Methods A 51-year-old woman from Germany with acute myeloid leukemia (AML) relapse after allogenic hematopoietic stem cell transplantation was treated with azacitidine and lenalidomide. She acquired an invasive fungal infection with mucor species *Lichtheimia ramosa* combined with *Aspergillus fumigatus* in functional pancytopenia. Three surgical pansinusrevisions were performed and high dose i.v. antifungal treatment with liposomal amphotericin B and isavuconazole was initiated. Due to missing treatment response with daily mucor progression nivolumab 240 mg was administered and complemented by interferon γ (100µg s.c. 5 doses). Administration was repeated every 2 weeks (in total 4 doses of nivolumab, but only 10 doses of interferon γ due to recurrent fever episodes) and simultaneously i.v. antifungal treatment was deescalated. Blood samples were collected before (baseline treatment (BT)) as well as 2 weeks (under treatment (UT) 1) and 5 weeks (UT2) after treatment initiation with nivolumab. Peripheral blood mononuclear cells were isolated and flow cytometry analyses of lymphocytic subsets were performed.

Results Ten days after first dose of nivolumab, long-term local hemostasis was achieved. Local symptoms disappeared, sinusitis complaints improved, and inflammation values decreased significantly. Sixteen days after treatment initiation a CT scan revealed a partial remission of mucormycosis invasion. Follow-up CT scans showed a stable disease. Expression of PD-1 on T cells was monitored as proof of concept from BT on and showed a significant reduction from 34.7% to 3.3% (UT1) and 1.38% (UT2). Both activation markers CD86 and CD69 showed an increase from BT to UT1. T cells showed high maturation markers throughout monitoring, while B cell maturation increased from BT to UT1/2. Nine

weeks after diagnosis and despite long-term neutropenia the patient was still clinically stable under nivolumab treatment and discharged with continued deescalated antimycotic treatment. A bone marrow biopsy revealed a further progression of AML relapse. After 3 weeks during follow-up mucormycosis was still clinically stable. Ten days later the patient developed fever up to 39.5°C, but refused to seek medical attention due to unfavorable prognosis of AML and died two days later from septic shock combined with disseminated intravascular coagulation.

Conclusions In immunocompromised hematological patients with invasive fungal infections, immune checkpoint inhibition is capable of reversing an infection-induced immunosuppressive phenotype. Therefore, it might complement the treatment of invasive fungal infections and should be evaluated in future clinical trials.

Disclosure Information N. Mueller: None. **J. Banck:** None. **S. Mellinghoff:** None. **H. Schlösser:** None. **M. Thelen:** None. **P. Koehler:** None. **F. Schrötzlmair:** None. **O. Cornely:** None. **L. H. Lindner:** None. **M. von Bergwelt-Baildon:** None.

P09.10 LOCAL IMMUNOTHERAPY OF BRAIN CANCER HARNESSING HIGH-RETENTION FC-FUSION CONSTRUCTS

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Background Glioblastoma is a highly aggressive cancer type and despite aggressive therapy, patients' survival remains poor. Immunotherapy of brain cancer is particularly difficult because of its location behind the blood-brain-barrier and the immunosuppressive tumour microenvironment. In order to (re-)activate the immune system, and reverse the local immunosuppression, we employ the pro-inflammatory cytokine interleukin 12 (IL-12). This highly potent immune-stimulatory agent is known for its anti-cancer effect. Unfortunately, IL-12 was found to induce severe toxicity when applied intravenously, impeding its way into clinics. Thus, currently the only valid therapeutic option is local application into the tumour site.

Materials and Methods Engineered proteins were expressed in HEK293T cells and purified by affinity chromatography. In vivo experiments were performed in glioma-bearing mice using intracranial injection of bioluminescent GL-261 cell line. Treatments were performed on day 21 and 28 post tumour injection through intracranial injection using a step-catheter modelling convection enhanced delivery in mice. Blood or tissue was analysed using immunohistochemistry, flow cytometry and ELISA.

Results Based on an IL-12-IgG fusion protein, we engineered a molecule for exclusively local therapy of brain cancer. We showed anti-cancer efficacy and increased tissue retention of the fusion molecule in glioma in mice. However, molecular analysis of treated tissue confirmed an upregulation of the immunosuppressive molecule PD-L1 in the tumour microenvironment. This means that, despite its efficacy, IL-12 induces an adaptive resistance mechanism, counteracting the therapeutic effect. We thus hypothesised that local IL-12 therapy combined with local blockade of the PD-1/PD-L1-axis would further improve therapeutic efficacy, while exclusively local administration would avoid increased side effects, which usually accompany combination immunotherapy. We showed significantly enhanced long-term survival of glioma-bearing mice treated with IL-12 therapy in combination with PD-L1 blockade compared to single or control treatments. In a next step, we engineered a novel, bifunctional molecule. Optimized for local application and minimized leakage into the systemic circulation, it combines immune-stimulation and checkpoint blockade in one entity. We showed anti-cancer efficacy and increased tissue retention in glioma in mice.

Conclusions The potent anti-cancer effect of the cytokine IL-12 can be used in therapy when applied locally into the brain tumour. Besides fusion to IgG, we introduced several specific modifications on the molecule, which are crucial to prevent systemic exposure and associated toxic side effects. To overcome the dampening of the immune reaction through induced PD-L1 expression, we introduced a combination therapy of IL-12 with a PD-L1-blocking antibody in a single molecule. We showed this combination superior to single treatments in the context of exclusively local brain tumour therapy.

Disclosure Information L. Schellhammer: None. **M. Beffinger:** None. **S. Pantelyushin:** None. **T. Buch:** None. **J. vom Berg:** None.

P09.11 TLR3 SUPPRESSES COLORECTAL CARCINOGENESIS, PRESUMABLY THROUGH UP-REGULATION OF T-CELL ATTRACTING CXC CHEMOKINES

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10.1136/jitc-2020-ITOC7.111

Background Toll-like-receptors (TLRs) are main components of the innate immune system which recognize endogenous or pathogen-associated molecular 'danger patterns'. Previous findings from us and others highlighted a role of TLRs in the formation of tumors. However, TLRs may have differing roles in immune and cancer cells, and the underlying mechanisms are still unclear. TLR downstream signaling is mediated by two adapter proteins; MyD88 (myeloid differentiation primary response gene 88) and TRIF (TIR-domain-containing adapter-inducing interferon-β). The MyD88-mediated signaling pathway is activated by all TLRs, except TLR3. We could show previously that it leads to the initiation of oncogenic, proliferative and pro-inflammatory responses in colorectal cancer. The endosomal receptor TLR3, in contrast, solely depends on TRIF. It recognizes viral, microbial and endogenous dsRNA leading to production of type-I interferon and chemokines, and induces apoptosis. The role of TRIF dependent TLR3 signaling in colorectal cancer is still disputed. Within this study, we show tumor-suppressive TLR3 functions prevailing over oncogenic effects in colorectal cancer.

Materials and Methods TLR3-deficient colon cancer cell lines were engineered by CRISPR-Cas9. Genetically modified mouse models were generated, based on a 'switch-on mutagenesis' approach, with global inactivation of Tlr3 or TRIF (Ticam1), allowing tissue specific re-expression based on Cre recombination. The mice were interbred with the Apc^{1638N} mouse model for digestive cancer. Furthermore, clinical significance of TLR3 expression levels was assessed in human colorectal cancer tissue samples from our clinic (n=81) and from TCGA datasets. A putative correlation between

intratumoral TLR3 expression and T-cell infiltration is currently analyzed.

Results Global deficiency of murine Tlr3, or TRIF, induced significantly increased digestive tumor formation, associated with increased morbidity indicating a tumor suppressive role. Coherently, TLR3 expression is highly significantly decreased in human colorectal cancer compared to normal mucosa, significantly correlated with poor survival. TLR3 deficient cell lines show reduced migration and slightly declined proliferation suggesting an oncogenic role on the cell-autonomous level. Nevertheless, gene expression analysis revealed that the dsRNA induced expression of T-cell attracting cytokines CXCL10 and CXCL11 in colon cancer cell lines is exclusively dependent on TLR3. These chemokines were shown to favor a TH1-type antitumoral response.

Conclusions TLR3 favors tumor suppression *in vivo*, presumably resulting from non-cell-autonomous factors such as the production of CXCL10 and 11 and resulting T-cell infiltration. This may outweigh the putative cell-autonomous oncogenic functions of TLR3 deficiency.

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P09.12 BIFUNCTIONAL SIRPα-CD123 FUSION ANTIBODY FOR THE ELIMINATION OF ACUTE MYELOID LEUKEMIA STEM CELLS

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Background Despite advances in the development of novel strategies against acute myeloid leukemia (AML), treatment options are limited and most patients relapse. Relapse occurs due to the persistence of chemotherapy-resistant leukemic stem cells (LSCs), which re-initiate the outgrowth of the disease, highlighting the need of targeting LSCs to improve patient survival. LSCs are characterized by the expression of the interleukin-3 receptor α, also known as CD123. CD123 is expressed on AML blasts and LSCs, and shows a moderate expression on normal hematopoietic stem cells, claiming CD123 as a suitable target antigen. CD47 is a ubiquitously expressed immune checkpoint upregulated on LSCs where it acts as an immune escape mechanism. CD47 transmits a 'don't eat me' signal upon its interaction with the signal regulatory protein alpha (SIRPα) receptor on macrophages, thus inhibiting phagocytosis. In order to efficiently eliminate LSCs, we have designed a bifunctional antibody that specifically targets CD123 and simultaneously blocks CD47. Importantly, our strategy restricts the benefits of the CD47 blockade to $CD123^+$ AML cells. Thus, we hypothesize a lower risk for on-target off-leukemia toxicity.

Materials and Methods The bifunctional SIRPα-CD123 antibody was generated by fusing an extracellular domain of the SIRPα receptor, which functions as the CD47 blocking domain, to the CD123 antibody. The biological activity of the SIRPα-CD123 antibody was examined using AML-derived MOLM-13 cells, primary AML patient material and patient-derived xenografted (PDX) AML cells with NOD.Cg-Prkdc^{scid} IL2rg^{tm1Wjl}/SzJ (NSG) mice.

Results The SIRPα fusion improved the binding of the bifunctional SIRPα-CD123 antibody to AML cells compared to a conventional CD123 antibody. The SIRPα-CD123 antibody enhanced the elimination of the AML-derived MOLM-13 cells by antibody-dependent cellular cytotoxicity via NK cells. Additionally, the cytotoxicity was confirmed using primary patient-derived AML cells. Furthermore, an improved cytotoxicity towards leukemia initiating AML PDX cells was observed with the SIRPα-CD123 antibody using luciferase bioluminescence *in vivo* imaging. With regards to the inhibition of CD47 signaling, we were able to show a blockade of CD47 on $CD123^+CD47^+$ cells by the SIRPα-CD123 antibody. Correspondingly, a significant increase in phagocytosis of primary patient-derived AML cells mediated by monocyte-derived macrophages was observed in both allogenic and autologous setting. We were also able to show a preferential binding to MOLM-13 in the presence of a 20-fold excess of red blood cells indicating a potential low on-target off-leukemia toxicity.

Conclusions The bifunctional SIRPα-CD123 fusion antibodies target the CD123+CD47+ cells and stimulate their phagocytosis by blocking the inhibitory CD47 signal. The dual mode of action of the SIRPα-CD123 has the potential to deplete the AML LSCs through NK cell cytotoxicity and macrophage-mediated phagocytosis while restricting the CD47 related on-target off-leukemia toxicity.

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P09.13 OPTIMIZATION OF A GMP-GRADE LARGE-SCALE EXPANSION PROTOCOL FOR CYTOKINE-INDUCED KILLER CELLS USING GAS-PERMEABLE STATIC CULTURE FLASKS

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Background 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.

Materials and Methods 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.

Results 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 10^9 total cells obtained per single well in 14 days, starting from just 2.5×10^6 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. 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.

Conclusions 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.

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P09.14 BLOCKING COUNTERREGULATION OF UNFOLDED PROTEIN RESPONSE BY TARGETED PROTEIN SYNTHESIS INHIBITION PRODUCES HIGHLY SYNERGISTIC CELL DEATH IN SEVERAL CANCER ENTITIES

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10.1136/jitc-2020-ITOC7.114

Background Because tumor cells have high proliferation rates the demand for energy on the one hand and proteins on the other hand is high. In line, protein folding machinery of the ER is heavily used. 2-Deoxyglucose (2-DG) not only blocks energy synthesis by inhibiting glycolysis but also blocks synthesis of mannosyl leading to impaired N-linked glycosylation, accumulation of misfolded proteins, and increased unfolded protein response (UPR). However, due to compensatory events, UPR-induced apoptosis is hampered. Therefore, we combined 2-DG with targeted protein synthesis inhibition by immunotoxins, consisting of an antibody and pseudomonas exotoxin, to enhance UPR mediated cell death.

Materials and Methods Established cell lines and patient-derived B-ALL samples were treated *in vitro* with various protein synthesis inhibitors and UPR-inducers. Drug synergy was determined mathematically as fold-increase over additivity. Biochemical studies were performed using western blots. *In vivo* enhancement was tested using systemic xenograft models.

Results The combination of Moxetumomab and 2-DG achieved a two to nine-fold synergy *in vitro*. Synergy was abrogated by the addition of Mannose suggesting UPR as cause of synergistic cell death. Similarly, Moxetumomab enhanced UPR-inducers Bortezomib and tunicamycin and protein synthesis inhibition by cycloheximide and puromycin enhanced 2-DG suggesting a conserved mechanism. Using HB21, an immunotoxin targeting human transferrin-receptor, breast cancer, hepatocellular carcinoma, and glioblastoma were sensitized to 2-DG induced cell death. Biochemically, 2-DG increased XBP-1-cleavage, expression of pro-apoptotic CHOP and of anti-apoptotic BIP. Moxetumomab, however, blocked the upregulation of BIP while maintaining CHOP correlating with synergistic increase in PARP-cleavage and apoptosis. In two systemic mouse models, bone marrow (BM) lymphoma infiltration was not reduced by 2-DG or tunicamycin alone but was reduced after treatment with Moxetumomab alone by 5-fold in the JeKo-1 and by 16-fold in the Ramos model, respectively. The combination of Moxetumomab and 2-DG achieved a three-fold synergy in the JeKo-1 model and achieved MRD-negative BM status in the Ramos model. Against patient-derived B-ALL of the Burkitt's type, 2-DG and Moxetumomab were up to 5-fold more active *in vitro* and up to 7-fold more active in mouse xenografts *in vivo*.

Conclusions Cell death after persisting unfolded protein response is synergistically enhanced by tumor-cell specific inhibition of protein synthesis against four distinct tumor entities at physiologically achievable concentrations. Our approach of immunotoxin-induced targeted protein synthesis inhibition opens a novel, so far undescribed therapeutic window which may warrant clinical evaluation.

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P09.15 TARGETING THE STROMA TO ENHANCE EFFECTOR MEMORY T CELL INFILTRATION AND ANTI-TUMOR RESPONSE TO ANTI-PD1 ANTIBODY IN PANCREATIC DUCTAL ADENOCARCINOMA

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10.1136/jitc-2020-ITOC7.115

Background Pancreatic ductal adenocarcinoma (PDAC) is resistant to immune checkpoint inhibition. One of the major resistance mechanisms is attributed to myeloid cells as an immunosuppressive element within the stroma of PDAC. It has been reported that focal adhesion kinase inhibitor (FAKi) can suppress immunosuppressive myeloid cells such as tumor associated macrophages (TAMs) and myeloid derived suppressor cells (MDSC), consequently sensitizing tumor to anti-PD1 antibody in mouse models of PDAC. Our group has previously shown in a murine model that targeting the stroma via PEGylated recombinant human hyaluronidase (PEGPH20) enhanced the anti-tumor activity of the whole cell vaccine (GVAX) by targeting CXCR4-expressing myeloid cells and led to an increase in infiltration of CCR7- effector memory T cell subsets. Here, we evaluate the hypothesis that FAK expressing myeloid cell subsets modulate T cell infiltration in human

PDAC and FAKi can synergize with PEGPH20 by targeting myeloid cells in PDAC.

Material and Methods Resected human PDAC tissue specimens treated with GVAX and anti-PD1 therapy was used to assess FAK expression in myeloid cell subsets and its impact on T cell infiltration. A sequential staining and stripping multiplex IHC technique that incorporates 28 myeloid and lymphoid biomarkers, as well as phosphorylated FAK (pFAK) combined with computational image processing was used to assess myeloid cell populations, T cell infiltration and FAK expression.

An established murine model of metastatic PDAC treated with and without anti-PD1 therapy was used to assess the synergy and immune-modulating effect of FAKi and stromal degradation of hyaluronan via PEGPH20.

Results In human PDAC, FAK is widely expressed in TAMs and neutrophils. Increased FAK expression is associated with increased CXCR4 expression. Lower pFAK density in neutrophils and M2 TAMs, but not lower pFAK density in M1 TAMs, is associated with higher CD8+ T cell infiltration.

FAKi and combination of FAKi with anti-PD1 extends survival in the mouse metastasis model of PDAC. Adding PEGPH20 to FAKi and anti-PD1 antibody significantly prolonged survival in this model. Comparing to the combination of FAKi and anti-PD1 antibody, adding PEGPH20 significantly decreased the number of CXCR4-expressing myeloid cells in the tumor microenvironment (TME) of PDAC and consequently led to an increase in the amount of CCR7+ central memory T cells. Additionally, the amount of G-MDSCs, inflammatory resident monocytes and PDL1 expressing myeloid cells in the TME of PDAC, was also decreased in PDAC treated with the triple combination of PEGPH20, FAKi and anti-PD1 antibody compared to FAKi and anti-PD1 antibody.

Conclusion FAK is widely expressed in myeloid cell populations, directly correlated with CXCR4 expression and decreased FAK expression in a myeloid (M2 TAMs, neutrophil) inflamed stroma is associated with infiltration of effector CD8 T cells in human PDAC. Stromal degradation of hyaluronan via PEGPH20 combined with FAKi and anti-PD1 antibody further depletes immunosuppressive cells in the TME including G-MDSCs, inflammatory resident monocytes and PDL1 expressing myeloid cells and appears to target the CXCR4 pathway through PEGPH20. These findings support testing the combination of FAKi and anti-PD1 antibody with agents targeting CXCR4 directly or indirectly by PEGPH20 in human PDAC.

Disclosure Information A. Osipov: None. **L. Zheng:** None.

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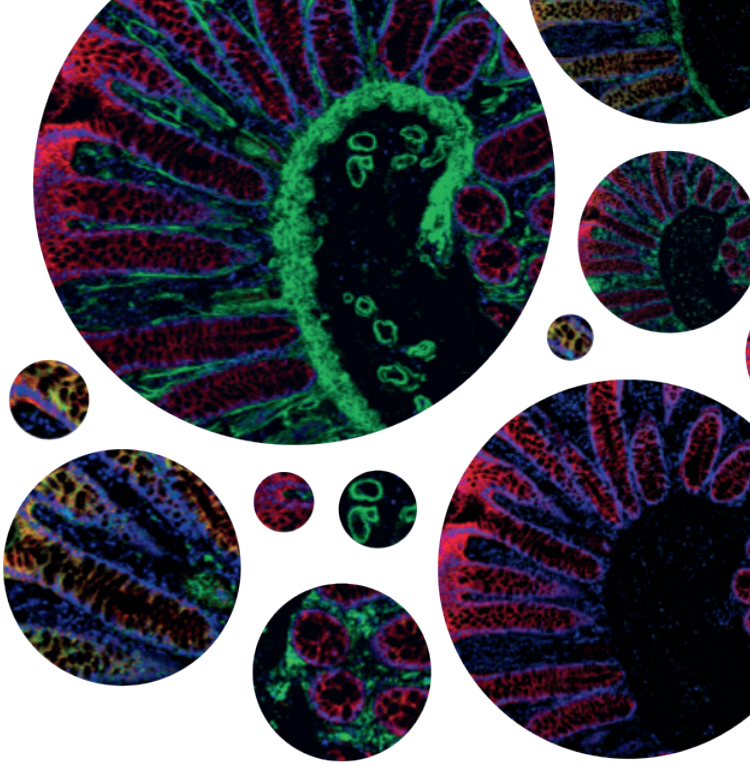

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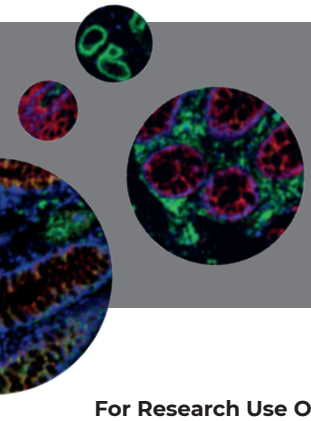

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