Journal for ImmunoTherapy of Cancer

Volume 9 Supplement 1

iTOC8 - THE 8TH LEADING INTERNATIONAL CANCER IMMUNOTHERAPY CONFERENCE IN EUROPE

8–9 October 2021 - VIRTUAL CONFERENCE

ABSTRACTS

BMJ

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ABSTRACTS

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Oral Presentations

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10.01 EFFECTIVE SOLID TUMOR THERAPY THROUGH ENHANCED RECRUITMENT AND IMMUNE SUPPRESSION SHIELDED T CELLS

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10.1136/jitc-2021-ITOC.1

Background CAR T cell therapy remains ineffective in solid tumors. Scarce T cell infiltration and T cell suppression at the tumor site are two notable therapy limitations. T regulatory (Treg) cells are capable of suppressing effective anti-tumor responses through inhibitory factors such as transforming growth factor β (TGF-β). Treg cells expressing the C-C chemokine receptor 8 (CCR8) have been found to accumulate and to correlate with poor prognosis in breast cancer. We postulated that CCR8 could be exploited to redirect effector T cells to the tumor site while a dominant-negative TGF-β receptor 2 (DNR) can simultaneously shield them from TGF-β.

Materials and Methods CCR8 and DNR can be expressed in murine and human T cells upon retroviral transduction. T cell receptor (TCR) and chimeric antigen receptor (CAR) antigen specific models in murine and human systems were utilized. qPCR, IF microscopy, ELISA and the cancer genome atlas (TCGA) database were used in the steps of ligand identification and hypothesis generation. We employed flow cytometry and multi-photon intra-vital microscopy to interrogate infiltration, proliferation and phenotype of T cell products. Mechanistically, CRISPR was used to dissect the role of the CCL1-CCR8 positive feedback loop in T cell therapy.

Results We identified that in an in vivo pancreatic murine model of cancer, the CCR8 gene was upregulated in tumor infiltrated lymphocytes compared to T cells that accumulated in the spleen. In this same tumor model, CCL1 could be detected in tumor explants. We identified that this secreted CCL1 from activated effector T cells potentiates a feedback loop for $CCR8^+$ T cell recruitment to the tumor site. The introduction of CCR8 and DNR receptors in primary T cells improved migration towards CCL1 and improved proliferation capacity in the presence of TGF-β. Besides these effects, these receptors did not further impact effector and memory phenotype or secretome of T cell products. The CCR8-driven sustained and improved infiltration synergized with TGF-β-shielding conferred by the DNR for improved therapeutic efficacy, allowing tumor rejection in models that are otherwise completely resistant to CAR T cell therapy.

Conclusions We conclude that the combination of CCR8- and DNR-transduction into antigen-specific T cells can exploit two critical biological axes to render T cell therapy effective in solid tumors such as pancreatic cancer. Beyond TGF-β, relieving other immunosuppressive axes may further sustain a CCL1 feedback loop mechanism to improve anti-tumoral function of CCR8+ ACT. This therapeutic approach could be extended to other Treg-rich solid tumor entities where limited infiltration into the tumor and intra-tumoral T cell proliferation prevent therapeutic success. Furthermore, the CCL1-CCR8 axis heralds the potential to be used as a target to improve the efficacy of immunotherapies beyond ACT.

Disclosure Information B.L. Cadilha: None. **M.R. Benmebarek:** None. **K. Dorman:** None. **A. Oner:** None. **T. Lorenzini:** None. **H. Obeck:** None. **S. Stoiber:** None. **D. Huynh:** None. **F. Märkl:** None. **M. Seifert:** None. **K. Manske:** None. **S. Endres:** None. **S. Kobold:** None.

10.02 GENOMIC HLA HOMOZYGOSITY IS FREQUENT IN ESOPHAGEAL ADENOCARCINOMA AND RELATED TO LOW IMMUNOGENICITY

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10.1136/jitc-2021-ITOC.2

Background Classical human leukocyte antigen (HLA) class I molecules are expressed by most somatic cells and present peptides to cytotoxic T cells. The HLA-genotype of an individual contains up to six different HLA-I molecules and defines the repertoire of peptides that can be presented to cytotoxic T cells. Homozygosity for one or more HLA-loci could translate in a smaller repertoire of tumour neoantigens possibly presented to cytotoxic T cells in an individual and potentially predispose such individuals with a disadvantage to fight a nascent tumour.

Material and Methods High-resolution HLA-genotyping from germline normal DNA of 80 esophago-gastric adenocarcinoma (EGA) patients was performed with the NGS method by Illumina. Whole exome sequencing (WES) was performed on tumor tissue and normal peripheral blood cells (n=39). The data were processed, and non-synonymous mutations were called. The amount of potential high-affinity binders derived from 10 cancer testis antigens (CTAs) frequently expressed in EGA and non-synonymous mutations obtained from WES data were determined using an in-silico approach for MHC-binding (IEDB.org). RNA-extraction and gene expression profiling were performed using the NanoString technology.

Results We compared the frequency of HLA homozygosity in EGA patients to an HLA-matched reference population derived from a large cohort of bone marrow donors (n=7.615 out of 615.017 donors). We demonstrate that EGA patients are more likely to be homozygous for at least one HLA-I gene than the control population. In EGA patients, 35% of HLA-A, -B, and -C alleles were homozygous in comparison with 19% of HLA alleles among the HLA-matched general population. This difference corresponded to an odds ratio (OR) for homozygosity of 2.282 (95% confidence interval (CI) 1.442-3.615, $p<0.001$). The odds ratios for homozygosity at HLA-A (OR=1.885, CI=1.111-3.236, $p<0.05$), HLA-B (OR=3.045, CI=1.346-6.499, $p<0.05$) and HLA-C (OR=2.170, CI=1.445-3.579, $p<0.05$) were significantly different. We then aimed to estimate the influence of HLA-homozygosity in the context of tumour immune surveillance. Predictions by IEDB analysis resource tool indeed showed a reduced repertoire of high and moderate-affinity MHC-

binders (both CTA-derived and mutation-derived peptides) in the homozygous cohort. Our findings demonstrate a reduced amount of potentially immunogenic peptides in EGA patients with HLA-homozygosity for at least one locus, which may result in impaired cancer immunosurveillance. In line with this observation, we also found increased levels of CTA expression in homozygous compared to heterozygous patients. After artificial modification of the genotype of homozygous patients to a heterozygous genotype, the set of predicted good-binding peptides was comparable to the heterozygous cohort.

Conclusion Our results highlight the effect of HLA-I homozygosity on the immunopeptidome as important prerequisite of anti-tumor immunity. The high frequency of genomic HLA-I homozygosity observed in the EGA cohort may reflect an increased cancer risk for these patients. Together with previous reports demonstrating reduced survival after checkpoint therapy, our study suggests consideration of germ-line HLA-homozygosity for the design and interpretation of immunotherapeutic trials.

Disclosure Information M.A. Garcia-Marquez: None. **M. Thelen:** None. **E. Bauer:** None. **K. Wennhold:** None. **J. Lehmann:** None. **D. Keller:** None. **B. Gathof:** None. **L. Maas:** None. **J. George:** None. **C. Bruns:** None. **A. Quaas:** None. **M. von Bergwelt-Baildon:** C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Modest; Astellas, Roche, MSD. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; BMS. **M. Peifer:** None. **H.A. Schlößer:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Astra Zeneca. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; BMS.

10.03 INTERLEUKIN-22 REGULATES ANTI-TUMOR IMMUNITY IN MOUSE MODELS OF LUNG AND BREAST CARCINOMA

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10.1136/jitc-2021-ITOC.3

Background High expression of CD155 (poliovirus receptor, PVR) is associated with a poor prognosis of lung adenocarcinoma (LUAD) and triple-negative breast cancer (TNBC) patients. When overexpressed, this molecule inhibits the antitumor function of NK and cytotoxic T cells through binding to its inhibitory co-receptors TIGIT and CD96, and downregulation of stimulatory CD226 (DNAM-1). However, the exact mechanism of CD155 overexpression on the tumor cells remains unclear. Here we demonstrate that interleukin-22 (IL-22), a cytokine known to promote cancer progression, induces upregulation of CD155 on tumor cells in mouse models of breast and lung cancer and may, thus, inhibit antitumor immunity and promote lung metastasis.

Materials and Methods To study the influence of IL-22 on antitumor immunity, we utilize IL-22-deficient animals in syngeneic mouse models of metastatic breast and lung cancer. For this purpose, we generated tumor cells deficient in IL-22 receptor (IL-22R) or in CD155 and tumor cells, that constantly express CD155 independent of its natural regulation. Here, we determine the incidence of metastasis and antitumor NK and T cell responses in the lung, the primary site of metastasis.

Results We demonstrate that murine cancer cells upregulate CD155 surface expression upon treatment with recombinant IL-22, whereas this effect is abolished in the absence of IL-22R. Furthermore, IL-22-deficient animals have a lower metastatic burden in the lung and demonstrate a dramatic increase in IFN-γ production in NK, and, to a lower extent, cytotoxic T cells. Moreover, this effect is reversed when CD155 is expressed on the tumor cells independent of its natural regulation, which enables lung metastases in IL-22 deficient animals. Phenotypically, NK cells in IL-22 knockout mice have a higher expression of co-stimulatory receptor CD226, which is linked to the antitumor potential of these cells.

Conclusions Here we demonstrate a novel pathway of cytokine-mediated cancer progression, where IL-22 is capable of inducing CD155 on the tumor cells and, therefore, promotes an immunosuppressive tumor microenvironment. This highlights the potential of IL-22 as a target for immunotherapy considering the complexity of the CD155-dependent immunoregulatory network.

Disclosure Information D. Briukhovetska: None. **J. Suarez-Gosalvez:** None. **M. Schübel:** None. **A. Markota:** None. **J. Jobst:** None. **J. Dörr:** None. **F. Märkl:** None. **M. Schwerdtfeger:** None. **A. Öner:** None. **M. Seifert:** None. **A. Gottschlich:** None. **S. Endres:** None. **S. Kobold:** None.

10.04 A LIBRARY OF NOVEL CANCER TESTIS SPECIFIC T-CELL RECEPTORS FOR T-CELL RECEPTOR GENE THERAPY

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10.1136/jitc-2021-ITOC.4

Background The positive clinical effect of T-cell receptor(TCR) gene therapy on tumor regression has previously been demonstrated by NY-ESO-1 TCR-gene therapy. To seriously increase the number of cancer patients that can be treated with TCR-gene therapy we aim to identify a novel set of high-affinity Cancer Testis (CT) specific TCRs targeting different CT-antigens in a variety of prevalent HLA-class I alleles.

Materials and Methods In this study, we selected by bioinformatic tools the most promising CT-genes to target, and from these genes we identified by HLA-peptidomics the naturally processed and presented HLA-class I peptides. With these peptides HLA-tetramers were generated, and by MACS enrichment and single cell sorting CT-specific $CD8^+$ T-cell clones were selected from the allo-HLA repertoire of healthy donors. By performing several different functional assays the high function avidity CT-clones with a safe recognition pattern were selected. To evaluate the potential for clinical application in TCR-gene therapy, TCRs were sequenced, and transferred into peripheral blood derived $CD8^+$ T cells.

Results In total we identified, 7 novel CT-specific TCRs that effectively target MAGE-A1, MAGE-A3, MAGE-A6 and MAGE-A9 expressing tumors cells in the context of HLA-A1, -A2, -A3, -B7, -C7 and -B35.

Conclusions With this set of 7 novel CT-specific TCRs we expand the arsenal of tumor specific TCRs. With this expanding library of TCRs it would be possible to select in future for each cancer patient, based on HLA typing and gene expression, a useful TCR to generate a personalized TCR-gene therapy products. In addition, patients could be treated with multiple TCRs to enhance the efficacy and increase the durability of clinical responses by reducing the likelihood of tumor escape.

Disclosure Information M.A.J. de Rooij: None. **D.M. Steen:** None. **D. Remst:** None. **A. Wouters:** None. **M.G.D. Kester:** None. **R.S. Hagedoorn:** None. **P.A. van Veelen:** None. **E.M.E. Verdegaal:** None. **J.H.F. Falkenburg:** None. **M.H.M. Heemskerk:** None.

Poster Presentations

P01 Emerging concepts/new agents

P01.01 SAFETY AND EFFICACY STUDY OF PEMBROLIZUMAB IN COMBINATION WITH LENVATINIB IN PARTICIPANTS WITH HEPATOCELLULAR CARCINOMA (HCC) BEFORE LIVER TRANSPLANT AS NEOADJUVANT THERAPY——PLENTY RANDOMIZED CLINICAL TRIAL

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10.1136/jitc-2021-ITOC.5

Background Patients with hepatocellular carcinoma (HCC) who exceed standard Milan criteria suffered from high post-transplant recurrence rate.This study will evaluate the safety and efficacy of pembrolizumab in combination with lenvatinib as neoadjuvant therapy in participants with HCC exceeding Milan criteria before liver transplant.

Materials and Methods Participates would be randomly assigned (1:1) to experimental or Comparator/Control by computer-generated allocation based on the envelope method and the hierarchical block randomization method(hierarchy: BCLC stage and AFP level). The envelopes are sealed opaque, and sequentially numbered.Randomization is performed by the trial coordinator.The random number table and the block assignment number table will be kept confidential by the full-time secretary of this project.Center-stratified block-permuted randomization is used in this trial. Then permuted block randomization is used for each stratum with a block size of 4.

Results The initial first patient was recruited in August.2020, the primary hypothesis of this study are that neoadjuvant pembrolizumab plus lenvatinib is superior to regularly waiting in the list with respect to: 1) recurrence-free survival (RFS) as assessed by blinded independent central review (BICR); and 2) Objective Response Rate (ORR).The investigators design a clinical study to explore whether the combination above as a neoadjuvant treatment in patients with advanced HCC before liver transplant could reduce postoperative recurrence and to analyze potential immune biomarker of therapeutic response.

Conclusions The study is still ongoing and the preliminary short term outcome was positive. HCC patients who exceeded milan criteria may benefit from neoadjuvant immunotherapy combined with TKI before liver transplantation.

Disclosure Information H. Feng: None. **Q. Xia:** None.

P01.02 TLR-MEDIATED SUPPRESSION OF THE CCL22-CCR4 AXIS AS A NEW TARGET FOR TUMOR IMMUNOTHERAPY

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10.1136/jitc-2021-ITOC.6

Background Unmethylated CpG-DNA is a potent ligand for the endosomal Toll-like-receptor-9, important for the immune activation to pathogen-associated molecules.[1] CpG and other TLR-ligands show effective immunotherapeutic capacities in cancer treatment by inducing an antitumorigenic immunity.[2] They are able to reduce tumor progression by reduction of intratumoral secretion of the immunoregulating chemokine CCL22[3] and subsequent recruitment of immunosuppressive regulatory T cells (Treg), which express CCR4 the only so far known receptor for CCL22.[4] Our recent work has shown that CCL22 secretion by dendritic cells (DC) in the lymph node, mediates tolerance by inducing DC-Treg contacts.[5] Indeed, in the absence of CCL22, immune responses to vaccination were stronger and resulted in tumor rejection.[6] Therefore, we are aiming to investigate the effects of TLR-ligands on systemic CCL22 levels, elucidating all involved mechanisms to identify new targets for cancer immunotherapy.

Materials and Methods T, B and $CD11c^+$ DCs of wildtype (wt) and $RAG1^{-/-}$ mice were isolated from splenocytes by magnetic-activated cell sorting for *in vitro* assays. Different co-cultures were incubated with CpG and GM-CSF, known as an CCL22 inducer.[5] For *in vivo* experiments, wt mice were treated with CpG, R484 or poly(I:C) alone and in combination with GM-CSF. CCL22-levels in a number of organs were analyzed.

Results Analyzing the different immune cell compartments *in vitro*, we found that DCs in whole splenocytes secrete CCL22 during culture while DC cultured alone showed no CCL22 secretion. When treated with CpG, CCL22-levels were reduced in splenocytes, while it was induced in DC culture alone. The same results were seen when RAG splenocytes, that lack functional B and T cells, were cultured with CpG. CpG treated B cells were able to suppress CCL22 secretion by DC unlike T cells alone. Co-cultures of T and B cells treated with CpG, however, induced the strongest CCL22 suppression in DC. *In vivo*, we could show that all TLR ligands tested reduced CCL22 in a number of organs significantly. Furthermore, CpG showed the strongest suppression of CCL22 even in the presence of the CCL22 inducer GM-CSF.[5]

Conclusions We could show that B cells with T cells mediate CCL22 suppression by TLR ligands. The fact that CpG was able to reduce CCL22 levels even in the presence of the inducer GM-CSF demonstrates the potent CCL22 suppressive capacity of TLR ligands.

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Disclosure Information J. Grün: None. **I. Piseddu:** None. **C. Perleberg:** None. **N. Röhrle:** None. **S. Endres:** None. **D. Anz:** None.

P01.03 TARGETING DIACLYGLYCEROL KINASE ALPHA AND ZETA BY SELF DELIVERING RNAI TO OPTIMIZE TLYMPHOCYTES FOR ADOPTIVE THERAPY OF SOLID TUMORS

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10.1136/jitc-2021-ITOC.7

Background Evidence indicates that diacylglycerol kinases (DGK) are promising targets for the optimization of T cell activity, for example in the setting of adoptive cell therapy (ACT). The tumor microenvironment (TME) of human renal cell carcinoma (RCC) is an immunosuppressive setting where T and NK cell functionality is blocked. DGK–α is a negative regulator of TCR signaling, functioning by metabolizing diacylglycerol to phosphatidic acid and thereby limiting the activation of MAPK/ERK1/2 signaling pathway. DGK-α is found increased in tumor-infiltrating lymphocytes (TIL) from RCC patients and also in adoptively transferred T cells after infiltrating into the TME.[1] We previously reported that inhibition of DGK-α restored functionality of unresponsive CD8 T cells and NK cells from RCC-TIL. Other studies demonstrated that knockdown or pharmacologic inhibition of DGK-α and DGK-ζ alone or together increased target cell killing and cytokine production, and protected T cells from inhibitory factors in the TME.[2] However, there are no inhibitors for DGK-ζ and available DGK-α inhibitors have undesired pharmacokinetic/pharmacodynamic properties and are highly toxic precluding their clinical application. Here, we present data using a novel RNA interference (RNAi) technology that can specifically target each DGK isoform.

Materials and Methods INTASYL™ compounds incorporate drug-like properties into RNAi, resulting not only in enhanced cellular uptake in the presence of serum but also eliminating the need for further transfection reagents. Toxicity of compounds applied alone or in combination was assessed by 7-AAD flow cytometry analysis and WST assay. Silencing of mRNA and protein was analyzed by RT-qPCR and SimpleWestern. Downstream signaling pathways and T cell function were analyzed to demonstrate pharmacological efficacy.

Results Two DGK-ζ compounds and one DGK-α compound were analyzed using Jurkat T cells and primary human TCR-transduced T cells. No effects were seen on cell viability for the compounds applied alone or in combination. On-target knockdown was achieved in Jurkat T cells evidenced by RT-qPCR and SimpleWestern. Silencing of mRNA and protein occurred quickly after 24h, peaked between 48h and 72h and lasted at least for 96h. Stimulation under DGK-targeting INTASYL treatment resulted in enhanced levels of phosphorylated ERK1/2 and enhanced secretion of IL-2.

Conclusions INTASYL™ self-delivering RNAi compounds represent a promising approach to target intracellular immune checkpoints such as DGKs. The good toxicity profile allows for combined application of several compounds enabling targeting of multiple checkpoints, which likely is necessary to counteract the complex and heterogeneous inhibitory influences of the TME. The technology enables the anti-tumor activity of T and NK cells for immunotherapy, and can be used in ACT and direct therapeutic applications towards the TME.

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Disclosure Information A.S. Herbstritt: C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Significant; Phio Pharmaceuticals. **M. Maxwell:** A. Employment (full or part-time); Significant; Phio Pharmaceuticals. **D. Yan:** A. Employment (full or part-time); Significant; Phio Pharmaceuticals. **B. Cuiffo:** A. Employment (full or part-time); Significant; Phio Pharmaceuticals. **J. Cardia:** A. Employment (full or part-time); Significant; Phio Pharmaceuticals. **S.P. Fricker:** A. Employment (full or part-time); Significant; Phio Pharmaceuticals. **E. Noessner:** C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Significant; Phio Pharmaceuticals.

P01.04 LENTIVIRAL PROTEIN VPX DELIVERY SYSTEMS AS POTENTIAL WEAPONS TO IMPROVE CYTARABINE TREATMENT RESPONSE AGAINST ACUTE MYELOID LEUKEMIA

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10.1136/jitc-2021-ITOC.8

Background Acute myeloid leukemia (AML) is an aggressive cancer of the blood, where malignant myeloid blasts accumulate in the bone marrow. One of the challenges of effective AML treatment is resistance to cytarabine (or ara-C), a standard AML chemotherapeutic drug used in front-line treatment today. In 2017, Schneider *et al.* reported the dNTPase sterile alpha motif and HD-domain-containing protein 1 (SAMHD1) to be a targetable biomarker for ara-C treatment response.[1] The intracellular triphosphorylated active form of ara-C, ara-CTP, was recognized as a substrate by SAMHD1 and is hydrolyzed back to ara-C. This led to a decrease in the amount of ara-CTP within the cells and consequently reduced cytotoxicity.[1] SAMHD1 can be targeted by the lentiviral accessory protein Vpx for proteasomal degradation by interacting with the proteasomal degradation complex and SAMHD1. This study aims to use Vpx to target SAMHD1 in AML cells to improve ara-C sensitivity.

Materials and Methods In order to manipulate SAMDH1 levels using Vpx, different Vpx delivery systems were developed. These are virus-like particles (VLPs) packaged with different homologs of Vpx from Simian Immunodeficiency Viruses (SIV) and HIV-2, and cell-penetrating peptides (CPPs) bound to either a 67 amino acid truncated SIVmac Vpx (67aaVpx) or to the WT full-length form. Two different CPPs were used in the synthesis: TAT and CPP44. The latter

was chosen, as significantly better uptake of the CPP was observed in AML cell lines and primary blasts compared to healthy PBMCs.[2].

Results Upon treating AML cell lines with the VLPs, we observed different SAMHD1-degradation capacities of the different Vpx homologs. SIVmac239 Vpx and HIV-2 7312a Vpx were most efficiently loaded into the VLPs, showed the highest SAMHD1-degradation and improved ara-C sensitivity up to 80-fold. In contrast, HIV-2 Rod9 Vpx did not show any SAMHD1 degradation or improvement in ara-C sensitivity despite its high packaging efficiency in the VLPs. As for the CPPs, CPP44 bound to 67aaVpx showed better uptake and SAMHD1 degradation compared to the TAT bound 67aaVpx in THP-1 cells, which is an AML cell line with high SAMHD1 expression levels. Upon co-treatment with ara-C, up to a 5-fold reduction in IC50 was observed when treated with CPP44-bound 67aaVpx. In order to increase the efficiency further, full-length Vpx-bound CPPs will be prepared, and trials using these CPPs are currently underway.

Conclusions

We demonstrate that inducing SAMHD1 degradation by Vpx delivered via VLPs or CPPs efficiently improved ara-C sensitivity in AML cell lines. Combining a Vpx delivery system with treatments containing ara-C might improve treatment outcomes in SAMHD1-high patients who are otherwise non-responsive.

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Disclosure Information R. Nair: None. **H. Baldauf:** None.

P01.05 DECIPHERING THE FUNCTION OF THE UBIQUITIN-PROTEASOME-SYSTEM IN REGULATING THE IMMUNE CHECKPOINT PROTEIN B7-H3 (CD276) IN NON-SMALL CELL LUNG CANCER

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10.1136/jitc-2021-ITOC.9

Background Cancer cells use the expression of immune checkpoint proteins on their surface to evade immune responses. Targeting these checkpoints with antibodies has substantially advanced cancer therapy in the past years, especially the treatment of lung cancer. However, the prognosis of metastatic lung cancer patients still remains poor and lung cancer remains to be the leading cause of cancer death worldwide. Further therapeutic concepts are therefore urgently needed.

It has been shown that protein expression levels of the immune checkpoint protein PD-L1, a member of the B7 protein family, is regulated by the ubiquitin-proteasome system (UPS). Ubiquitin-ligases (E3-ligases) and deubiquitinating enzymes that regulate immune checkpoint levels on the cell surface are therefore considered promising potential drug targets. Inhibiting enzymes that increase immune checkpoint surface levels might increase the anti-cancer immune response.

Here, we investigate whether another B7 family member, immune checkpoint protein B7-H3, is regulated by the UPS in non-small cell lung cancer (NSCLC).

Materials and Methods B7-H3 expression in NSCLC cell lines and patient samples was evaluated using mRNASeq data from open databases. Immunoblotting and FACS were used to analyse total endogenous protein levels and surface expression of B7-H3 in different NSCLC lines under normal growth conditions and in response to various inhibitors (MG-132, Chloroquine (CQ) and Cycloheximide (CHX)). Immunoprecipitation of FLAG-tagged B7-H3 followed by a TUBE IP using ubiquitin-binding beads and in-vivo ubiquitylation assays based on co-overexpression of HA-tagged ubiquitin and/or HA-tagged K48/K63-linkages specific ubiquitin together with FLAG-tagged B7-H3 or FLAG-tagged B7-H3 K526R mutant in HEK-93T cells were performed to analyse ubiquitination on B7-H3. Mass spectrometry analysis of FLAG-purified B7-H3 was performed to identify possible interaction partners.

Results Database analysis revealed that B7-H3 expression is higher in lung cancer samples than in healthy lung tissue. We found that B7-H3 is highly expressed in different NSCLC lines on RNA and protein levels. Treatments with either proteasomal (MG-132) or lysosomal (CQ) degradation inhibitors alone showed only minor effects on B7-H3 protein abundance. However, CHX treatment of H1437 cells decreased B7-H3 over time and this decrease was recovered by adding MG-132 or CQ suggesting that both the lysosome as well as proteasome are involved in the degradation of B7-H3. In vivo ubiquitination and TUBE assay showed K48 and K63 B7-H3 ubiquitination. Mass spectrometry analysis of FLAG-tagged purified B7-H3 revealed E3-ligase Trim21, which has recently been identified as a ligase of PD-L1 in lung cancer lines, as a potential interaction partner. Further experiments are planned to validate the result and to identify other UPS-related enzymes involved in post-translational B7-H3 surface level regulation.

Conclusions Our experiments indicate that immune checkpoint B7-H3 levels are regulated by the ubiquitin-proteasome system in NSCLC lines. With further experiments, we aim to identify UPS-related enzymes that stabilize B7-H3 on the cell surface. Pharmacological inhibition of such enzymes might reduce the immune checkpoint's surface levels and increase anti-tumour immune responses.

Disclosure Information M. Kurz: None. **L. Rieger:** None. **P. Giansanti:** None. **B. Kuster:** None. **F. Bassermann:** None.

P01.06 OVERWEIGHT AND OBESITY AS BIOMARKERS FOR SURVIVAL OUTCOMES AND IMMUNE RELATED ADVERSE EVENTS UNDERGOING IMMUNOTHERAPY – A SYSTEMATIC REVIEW AND META-ANALYSIS

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10.1136/jitc-2021-ITOC.10

Background The impact of overweight/obesity in cancer patients treated with immune checkpoint inhibitors (ICIs) is controversial. To further contribute to this debate, we

performed a systematic review and meta-analysis of published articles evaluating the effects of overweight/obesity on survival and immune-related adverse events (irAEs).

Materials and Methods In analogy to Cochrane recommendations, systematic literature searches included all published articles in PubMed until February 2021 with key terms 'obesity' and 'overweight' and ICI treatment irrespective of cancer entity and ICI used. Further selection criteria for meta-analysis included WHO cut-offs for overweight/obesity. For the random effects meta-analysis, we used Hazard Ratios (HR) for overall and progression-free survival (OS, PFS) and Odds Ratios (OR) for occurrence of irAEs with corresponding 95% confidence intervals (95%CI), respectively.

Results A total of 30 studies (12,895 patients, 38% female) selected for meta-analysis revealed a superior survival of overweight/obese patients (PFS: HR 0.9, 95%CI 0.77-1.04, p = 0.11; OS: 0.74, 95%CI 0.63-0.92, p = 0.0005) compared to normal weight patients. In subgroup analyses based on sex, overweight/obese male patients showed increased survival (PFS: HR 0.79, 95%CI 0.63-1.00, p = 0.05; OS: 0.71, 95% CI 0.58-0.86, p = 0.0005), whereas overweight/obese female patients had similar survival probabilities compared to their normal weight counterparts (PFS: HR 1.01, 95%CI 0.69-1.47, p = 0.96; OS: HR 0.73, 95%CI 0.48-1.10, p = 0.13). Underweight patients showed inferior survival (PFS: HR 1.48, 95%CI 1.07-2.04, p = 0.02; OS: HR 1.86, 95%CI 1.13-3.04, p = 0.01). In addition, overweight/obese patients had a higher risk of developing irAEs with grade $\geq$ 3 (OR 1.91, 95%CI 1.18-3.10, p = 0.008).

Conclusions Our meta-analysis revealed that overweight/obesity is a beneficial factor for PFS and OS in a mixed cohort of cancer patients undergoing ICI treatment accompanied by an increased risk of severe irAEs. The differences between overweight/obese males and overweight/obese females might point to sex specific adipose distribution patterns and interactions of sex steroids on a molecular level. A significant number of studies included underweight patients into normal weight control groups which led to a compromised interpretation of the data and should be addressed in future studies.

Disclosure Information P. Trinkner: None. **S. Günther:** None. **M. von Bergwelt:** None. **D. Cordas dos Santos:** None. **S. Theurich:** None.

P01.07 DEPLETION OF CD206^{HIGH} TUMOUR-ASSOCIATED MACROPHAGES USING A NANOCONJUGATE LIMITS TUMOUR BURDEN & DISSEMINATION IN METASTATIC TRIPLE NEGATIVE BREAST CANCER IN MICE

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10.1136/jitc-2021-ITOC.11

Background Anti-inflammatory (M2) tumour-associated macrophages (TAMs) exert protumoural roles through angiogenesis, immunosuppression and resistance to therapies.[1] M2 TAMs express the mannose receptor, CD206,[2] excellent marker for targeted therapies. We have previously identified a peptide called mUNO[2] that specifically binds to CD206 on M2 TAMs. Aiming to dissect the role of CD206^{high} M2 TAMs in the tumour progression and immunosuppression, we depleted them using an mUNO and doxorubicin (Adriamycin®)-containing polymer-drug nanoconjugate (St-PGA-DOX-mUNO, 'OximUNO') where the polymer backbone is branched polyglutamic acid (St-PGA).[3]

Materials and Methods We compared OximUNO with free DOX and the untargeted nanoconjugate St-PGA-DOX. To study the in vitro cytotoxicity of the nanoconjugates, we used M2 and M1 skewed macrophages derived from human blood buffy coat. To study the in vivo homing of nanoconjugates we used an orthotopic triple negative breast cancer (TNBC, 4T1 cells) model and a TNBC experimental metastases model in immunocompetent mice. For in vivo therapeutic efficacy studies, we used orthotopic and experimental metastases models of TNBC, and administered the compounds intraperitoneally (i.p.).

Results In vitro, OximUNO showed 39% higher toxicity to the primary human M2 macrophages than St-PGA-DOX, and 31% lower toxicity to the M1 macrophages than St-PGA-DOX. In vivo, OximUNO showed no change in creatinine or alanine aminotransferase values, indicating no toxic effects to the kidneys or liver. Compared to control St-PGA, i.p.-administered St-PGA-mUNO, showed improved homing to M2 TAMs in both orthotopic and experimental metastases models with low accumulation in the liver. In the orthotopic treatment study, only OximUNO significantly reduced the tumour volume and showed 56% and 38% less lung metastases than DOX and St-PGA-DOX, respectively. Additionally, DOX and St-PGA-DOX produced a significant bodyweight loss whereas OximUNO did not. Importantly, OximUNO treatment resulted in 2-5-fold increase in the ratio of $CD8^+/FOXP3^+$ expression, suggesting a shift in the immune landscape towards an immunostimulatory profile. In the experimental metastases model, OximUNO monotherapy resulted in the highest reduction of lung metastases, and this effect correlated with a significant reduction in CD206^{high} M2 TAMs; whereas no significant effect on M2 TAMs population was observed with DOX or untargeted nanoconjugate.

Conclusions Our data suggests that the elimination of CD206^{high} M2 TAMs with OximUNO suppresses spontaneous and experimental metastases in safe manner, shifts immune landscape towards immunostimulatory and could therefore be a potential treatment option for TNBC patients.

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Disclosure Information A. Lepland: None. **A. Malfanti:** None. **U. Haljasorg:** None. **S. Dordevic:** None. **L. Salumäe:** None. **P. Peterson:** None. **T. Teesalu:** None. **M.J. Vicent:** None. **P. Scodeller:** None.

P01.08 SARCOPENIA AS BIOMARKER FOR IMMUNOTHERAPY OUTCOMES AND IMMUNE-RELATED ADVERSE EVENTS – A SYSTEMATIC REVIEW AND META-ANALYSIS

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10.1136/jitc-2021-ITOC.12

Background Sarcopenia is an established risk factor for oncologic treatments like surgical interventions and conventional chemotherapy. However, the impact of sarcopenia on treatment and immune-related adverse events (irAEs) of cancer patients treated with immune checkpoint inhibitors (ICIs) continues to be debated. Therefore, we performed a systematic review and meta-analysis of all published articles evaluating the effects of sarcopenia on survival outcomes and irAEs of patients undergoing ICI treatment.

Materials and Methods In analogy to the Cochrane guidelines for systematic reviews, we performed a systematic literature search including all published articles in PubMed until February 2021 for the key terms 'sarcopenia' or 'sarcopenic obesity' in combination with several terms for ICI treatments, irrespective of cancer entity and ICI used. Further selection criteria for meta-analysis included defined cut-offs for sarcopenia. Reported outcomes included progression-free survival (PFS), overall survival (OS) and the frequency of irAEs. For the random effects meta-analysis, we used Hazard Ratios (HR) for OS and PFS and Odds Ratios (OR) for occurrence of irAEs with corresponding 95% confidence intervals (95%CI), respectively.

Results A total of 15 studies with 1,428 patients were selected to be eligible for meta-analysis. To evaluate muscle mass, all studies used CT-derived body composition parameters at the third lumbar vertebrae level and defined sarcopenia by using skeletal muscle index (SMI), psoas muscle index (PMI) or skeletal muscle density (SMD). Sarcopenic patients showed an inferior survival compared to non-sarcopenic patients with a combined HR for PFS with 1.53 (95%CI 1.23-1.91, p= 0.0001) and for OS with 1.6 (95% CI 1.23-2.09, p= 0,0005). Frequency of irAEs did not differ between sarcopenic and non-sarcopenic patients regardless of irAE grade (irAEs of grade$\geq$3: OR 1.14, 95%CI 0.65-2.01, p = 0.64, irAEs of any grade: OR 0.96, 95%CI 0.65-1.42, p = 0.85).

Conclusions This is the first meta-analysis that assessed sarcopenia in a mixed cohort of cancer patients. It revealed that sarcopenia is an adverse risk factor for survival of patients undergoing ICI treatment without affecting the risk of developing irAEs. Future studies may address sarcopenia as a patient-derived risk factor emphasizing the importance of nutrition and physical activity interventions.

Disclosure Information S. Günther: None. **P. Trinkner:** None. **M. von Bergwelt:** None. **D. Cordas dos Santos:** None. **S. Theurich:** None.

P02 Tumor microenvironment and microbiome in Immunotherapy

P02.01 T- AND B-CELL ABUNDANCE ARE REMARKABLY REDUCED IN THE IMMUNE MICROENVIRONMENT OF POST-TRANSPLANT MALIGNANCIES

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10.1136/jitc-2021-ITOC.13

Background Immunosuppressive medication is mandatory in the majority of solid organ transplant recipients to reduce the risk of allograft rejection. An increased risk to develop cancer is a negative side effect of long-term immunosuppression and impaired cancer immunosurveillance is assumed as underlying mechanism. However, the impact of immunosuppression on the tumor immune microenvironment (TME) is poorly understood. In this study we aimed to elucidate differences between immune infiltrates of post-transplant malignancies and cancer of non-immunosuppressed patients.

Materials and Methods 117 resected tumor samples of 80 organ transplant (kidney, heart, lung and liver) recipients were included. Immunohistochemistry and digital image analysis of whole section slides was used to quantify T- (CD3, CD8) and B-cell (CD20) abundance in the TME of 14 different cancer types. These data were used to calculate the Immune-score and to quantify tertiary lymphoid structures in the TME. Expression of Human-Leucocyte-Antigen-I (HLA-I) and programmed cell death ligand 1 (PD-L1) were analyzed in tissue microarrays. Clinical parameters were included in statistical analyses.

Results The increased risk of cancer in organ transplant recipients was reflected by a remarkably reduced immune infiltrate in the central region (CT) and the surrounding tissue (invasive margin, IM) of cancer areas. T cell abundance was decreased in IM and CT of skin (814 vs. 1440 $CD3^+$ cells/mm^2, $p < 0.01$) and non-skin tumors (479 vs. 781 $CD3^+$ cells/mm^2, $p < 0.01$), when compared to non-immunosuppressed controls. These differences were more pronounced in the IM than in the CT and larger when comparing abundance of $CD8^+$ T cells. The Immune-score integrating results from CT and IM was also decreased in transplant recipients. Similar to the results observed for T cells, B cell abundance and density of tertiary lymphoid structures were lower in cancer samples of transplant recipients. Decreased expression of HLA-I was more common in transplant recipients whereas the fraction of samples with PD-L1 expression was higher in controls.

Conclusions Our study demonstrates that post-transplant malignancies show a low immune infiltrate and supports the hypothesis of reduced anti-tumor immune response as an important mechanism underlying increased risk of cancer in organ transplant recipients. Optimized immunosuppressive protocols may be able to reduce cancer incidence and cancer therapies need to consider the distinct immune microenvironment of post-transplant malignancies.

Disclosure Information S. Schran: None. **R. Datta:** None. **O. Persa:** None. **C. Aguilar:** None. **M. Thelen:** None. **J. Lehmann:** None. **M. Garcia-Marquez:** None. **K. Wennhold:** None. **A. Quaas:** None. **C. Bruns:** None. **C. Mauch:** None. **H. Löser:** None. **D. Stippel:** None. **H. Schlößer:** None.

P02.02 SINGLE-CELL RNA SEQUENCING OF NEUROBLASTOMA TUMORS REVEALS IMMUNOREGULATORY INTERACTIONS AS NOVEL TARGETS FOR IMMUNOTHERAPY

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10.1136/jitc-2021-ITOC.14

Background Immunotherapy with CAR-T cells, as well as immune checkpoint blockade, show limited clinical efficacy in the pediatric solid cancer neuroblastoma, despite the success in various adult cancers. The lacking efficacy may be due to various immune evasion strategies employed by neuroblastoma tumors, leading to altered functionality of tumor-infiltrating immune cells. We aimed to provide a comprehensive overview of the composition and function of the neuroblastoma immune environment, as well as relevant immunoregulatory interactions (=), to identify novel targets for immunotherapy.
Materials and Methods 25 tumor samples from 20 patients (17 with high-risk disease, 6 with MYCN amplification), were collected during diagnostic biopsy pre-treatment (n=10) or during resection surgery after induction chemotherapy (n=15). Samples were enzymatically digested, single-cell FACS sorted and sequenced by Cel-Seq2 protocol.
Results Lymphoid cells in the TME consisted of αβ-, γδ-T cells, NK cells and B cells. Among αβ-T cells we identified $CD8^+$ T cells, two functionally distinct clusters of $CD4^+$ T cells, naive-like T cells and $FOXP3^+$ regulatory T cells (Tregs). $CD8^+$ T cells had reduced cytotoxic capacity compared to blood-derived T cells from a reference group. Tregs expressed high levels of *PRDM1*, *LAYN* and *ICOS*, suggesting an effector Treg profile, which is associated with increased inhibitory capacity. Although NK cells expressed the cytotoxic genes *NKG7*, *KLRF1*, *GNLY*, *GZMB* and *PRF1*, their expression was significantly lower than in blood-derived reference NK cells. Gene set enrichment analysis (GSEA) confirmed a reduced cytotoxic capacity of tumoral NK cells, which correlated with a decreased expression of activating receptors ($r=0.41$, $p<0.001$) and increased TGFβ signaling ($r=-0.45$, $p<0.001$). In addition, NK cells highly expressed the heterodimeric receptor *KLRC1:KLRD1*, which can inhibit NK cell function through HLA-E binding. High *HLA-E* expression by endothelial, immune and mesenchymal cells confirmed its inhibitory activity in the TME. Within the myeloid compartment we identified various immunosuppressive populations, comprising a cluster of *IL10* and *VEGFA* expressing macrophages, three clusters of M2 differentiated macrophages expressing *MMP9* and *LGALS3*, and dendritic cells with intact antigen presenting capacity, but high expression of numerous genes encoding immunosuppressive molecules such as *IDO1*, *LGALS1*, *LGALS2*, *CCL22* and *NECTIN2*. In MYCN amplified tumors, specifically, we observed even lower cytotoxic capacity of $CD8^+$ T and NK cells. We identified increased TGFB1 expression and defective antigen presentation by myeloid and tumor cells as potential causes for reduced cytotoxicity in MYCN amplified tumors. To identify relevant targets for immunotherapy we constructed an unbiased interaction network, which revealed *NECTIN1=CD96* and *MIF=CD74* as active immunoregulatory interactions between tumor and T/NK cells, and *CD80/CD86=CTLA4*, *CLEC2D=KLRB1*, *HLA-E=KLRC1/KLRC2*, *CD99=PILRA*, *LGALS9=HAVCR2*, and *NECTIN2=-TIGIT* between myeloid and T/NK cells.
Conclusions Cytotoxic lymphocytes in the neuroblastoma TME show reduced cytotoxic capacity, likely due to highly immunosuppressive myeloid cells, Tregs and numerous immunoregulatory interactions, which may serve as novel targets for immunotherapy in neuroblastoma.

Disclosure Information J. Wienke: None. **W.M. Kholosy:** None. **L.L. Visser:** None. **K.M. Keller:** None. **P. Lijnzaad:** None. **T. Margaritis:** None. **K.P.S. Langenberg:** None. **R.R. De Krijger:** None. **F.C.P. Holstege:** None. **J.J. Molenaar:** None.

P02.03 AUTOMATED CELL TYPE SPECIFIC PD-L1 QUANTIFICATION BY ARTIFICIAL INTELLIGENCE USING HIGH THROUGHPUT BLEACH & STAIN 15-MARKER MULTIPLEX FLUORESCENCE IMMUNOHISTOCHEMISTRY IN HUMAN CANCERS

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10.1136/jitc-2021-ITOC.15

Background The quantification of PD-L1 (programmed cell death ligand 1) has been used to predict patient's survival, to characterize the tumor immune microenvironment, and to predict response to immune checkpoint therapies. However, a framework to assess the PD-L1 status with a high interobserver reproducibility on tumor cells and different types of immune cells has yet to be established.
Materials and Methods To study the impact of PD-L1 expression on the tumor immune microenvironment and patient outcome, a framework for fully automated PD-L1 quantification on tumor cells and immune cells was established and validated. Automated PD-L1 quantification was facilitated by incorporating three different deep learning steps for the analysis of more than 80 different neoplasms from more than 10'000 tumor specimens using a bleach & stain 15-marker multiplex fluorescence immunohistochemistry panel (i.e., PD-L1, PD-1, CTLA-4, panCK, CD68, CD163, CD11c, iNOS, CD3, CD8, CD4, FOXP3, CD20, Ki67, CD31). Clinicopathological parameter were available for more than 30 tumor entities and overall survival data were available for 1517 breast cancer specimens.
Results Comparing the automated deep-learning based PD-L1 quantification with conventional brightfield PD-L1 data revealed a high concordance in tumor cells ($p<0.0001$) as well as immune cells ($p<0.0001$) and an accuracy of the automated PD-L1 quantification ranging from 90% to 95.2%. Across all tumor entities, the PD-L1 expression level was significantly higher in distinct macrophage/dendritic cell (DC) subsets (identified by CD68, CD163, CD11c, iNOS; $p<000.1$) and in macrophages/DCs located in the Stroma ($p<0.0001$) as compared to intratumoral macrophages/DC subsets. Across all different tumor entities, the PD-L1

expression was highly variable and distinct PD-L1 driven immune phenotypes were identified based on the PD-L1 intensity on both tumor and immune cells, the distance between non-exhausted T-cell subsets (i.e. PD-1 and CTLA-4 expression on $CD3^+CD8^+$ cytotoxic T-cells, $CD3^+CD4^+$ T-helper cells, $CD3^+CD4^+FOXP3^+$ regulatory T-cells) and tumor cells as well as macrophage/(DC) subtypes. In breast cancer, the PD-L1 fluorescence intensity on tumor cells showed a significantly higher predictive performance for overall survival with an area under receiver operating curves (AUC) of 0.72 ($p<0.0001$) than the percentage of $PD\text{-}L1^+$ tumor cells (AUC: 0.54). In PD-L1 positive as well as negative breast cancers a close spatial relationship between T- cell subsets ($CD3^+CD4^{\pm}CD8^{\pm}FOXP3^{\pm}PD\text{-}1^{\pm}CTLA\text{-}4^{\pm}$) and Macrophage/DC subsets ($CD68^{\pm}CD163^{\pm}CD11c^{\pm}$iNOS) was found prognostic relevant ($p<0.0001$).

Conclusions In conclusion, multiplex immunofluorescence PD-L1 assessment provides cutoff-free/continuous PD-L1 data which are superior to the conventional percentage of $PD\text{-}L1^+$ tumor cells and of high prognostic relevance. The combined analysis of spatial PD-L1/PD-1 data and more than 20 different immune cell subtypes of the immune tumor microenvironment revealed distinct PD-L1 immune phenotypes.

Disclosure Information N.C. Blessin: None. **E. Bady:** None. **T. Mandelkow:** None. **C. Yang:** None. **J. Raedler:** None. **R. Simon:** None. **C. Fraune:** None. **M. Lennartz:** None. **S. Minner:** None. **E. Burandt:** None. **D. Höflmayer:** None. **G. Sauter:** None. **S.A. Weidemann:** None.

P02.04 TISSUE-INFILTRATING TH9 CELLS IN HUMAN ENDOMETRIAL CANCER

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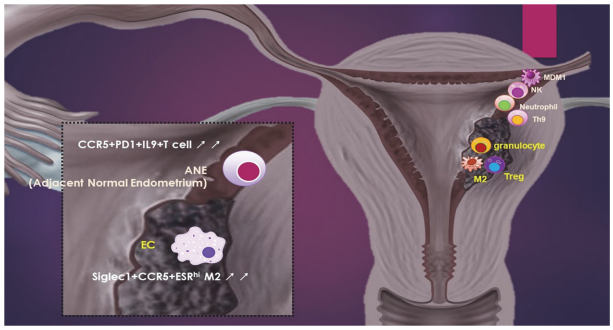
10.1136/jitc-2021-ITOC.16

Background Endometrial cancer (EC) is a hormone-related carcinoma with increased morbidity among female patients of all backgrounds. The immune microenvironment of EC is uncertain.

Materials and Methods 102 patients were recruited in the present study. 90 postoperative specimens from the patients were analyzed by immunohistochemistry. The leukocyte landscape of endometrial cancer was mapped using high-dimensional single-cell profiling (CyTOF) for 12 patients.



Abstract P02.04 Figure 1

Results NK cells, MDMs, and neutrophils were enriched in adjacent normal tissue. CCR5+CD38+ PD1+Th9 cells were enriched in the invasive margin. Additionally, $PD1{+}ESR_{neg}$ T cells and $Siglec1{+}CCR5{+}CD40{+}ESR^{hi}$ macrophage were infiltrated in the tumors.

Conclusions Immunological landscape of EC might shed light on new immunotherapuetic approach.

Disclosure Information H. Tong: None. **H. Feng:** None. **X. Wan:** None.

P02.05 COMPREHENSIVE PROFILING OF TUMOR HETEROGENEITY AND ITS MICROENVIRONMENT IN ADVANCED NON-SMALL CELL LUNG CANCER AT SINGLE CELL RESOLUTION

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10.1136/jitc-2021-ITOC.17

Background Lung cancer is a highly heterogeneous disease. Cancer cells and cells within the tumor microenvironment together determine disease progression, as well as response to or escape from treatment.

Materials and Methods To map the cell type-specific transcriptome landscape of cancer cells and their tumor microenvironment in advanced non-small cell lung cancer (NSCLC), we analyzed 42 tissue biopsy samples from stage III/IV NSCLC patients by single cell RNA sequencing and presented the large scale, single cell resolution profiles of advanced NSCLCs.

Results In addition to cell types described in previous single cell studies of early stage lung cancer, we were able to identify new cell types such as follicular dendritic cells and T helper 17 cells. Tumors from different patients display large heterogeneity in cellular composition, chromosomal structure, developmental trajectory, intercellular signaling network and phenotype dominance. Our study also revealed a correlation of tumor heterogeneity with tumor associated neutrophils, which might help to shed light on their function in NSCLC.

Conclusions This study presented first-time the tumor heterogeneity and tumor microenvironment profile from late-stage, largely untreated NSCLC patients, and shed light on possible treatment regimes.

Disclosure Information Y. Cordts: A. Employment (full or part-time); Modest; Singleron Biotechnologies GmbH. **F. Wu:** None. **J. Fan:** A. Employment (full or part-time); Modest; Singleron Biotechnologies GmbH. **M. Odenthal:** None. **R. Buettner:** None. **C. Zhou:** None.

P02.06 SEMI-AUTOMATED VALIDATION AND QUANTIFICATION OF CTLA-4 IN 90 DIFFERENT TUMOR ENTITIES USING MULTIPLE ANTIBODIES AND ARTIFICIAL INTELLIGENCE

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10.1136/jitc-2021-ITOC.18

Background CTLA-4 is an inhibitory immune checkpoint receptor and a negative regulator of anti-tumor T-cell

function. This study aimed at a comparative analysis of CTLA-4$^+$ cells between different tumor entities.

Materials and Methods To quantify CTLA-4$^+$ cells, 4,582 tumor samples from 90 different tumor entities as well as 608 samples of 76 different normal tissue types were analyzed by immunohistochemistry in a tissue microarray format. Two different antibody clones (MSVA-152R and CAL49) were validated and quantified using a deep learning framework for automated exclusion of unspecific immunostaining.

Results Comparing both CTLA-4 antibodies revealed a clone dependent unspecific staining pattern in adrenal cortical adenoma (63%) for MSVA-152R and in pheochromocytoma (67%) as well as hepatocellular carcinoma (36%) for CAL49. After automated exclusion of non-specific staining reaction (3.6%), a strong correlation was observed for the densities of CTLA-4$^+$ lymphocytes obtained by both antibodies ($r=0.87$; $p<0.0001$). The mean density of CTLA-4$^+$ cells was 674 $\pm$1482 cells/mm^2 and ranged from 71$\pm$175 cells/mm^2 in leiomyoma to 5916$\pm$3826 cells/mm^2 in Hodgkin's lymphoma. Within epithelial tumors, the density of CTLA-4$^+$ lymphocytes were higher in squamous cell (421$\pm$467 cells/mm^2) and urothelial carcinomas (419$\pm$347 cells/mm^2) than in adenocarcinomas (269$\pm$375 cells/mm^2) and renal cell neoplasms (256$\pm$269 cells/mm^2). A high CTLA-4$^+$ cell density was linked to low pT category ($p<0.0001$), absent lymph node metastases ($p=0.0354$), and PD-L1 expression in tumor cells or inflammatory cells ($p<0.0001$ each). A high CTLA-4/CD3-ratio was linked to absent lymph node metastases ($p=0.0295$) and to PD-L1 positivity on immune cells ($p<0.0026$).

Conclusions Marked differences exist in the number of CTLA-4+ lymphocytes between tumors. Analyzing two independent antibodies by a deep learning framework can facilitate automated quantification of immunohistochemically analyzed target proteins such as CTLA-4.

Disclosure Information D. Dum: None. **T.L.C. Henke:** None. **T. Mandelkow:** None. **E. Bady:** None. **R. Simon:** None. **G. Sauter:** None. **S. Steuerer:** None. **W. Wilczak:** None. **E. Burandt:** None. **J. Raedler:** None. **M. Lennartz:** None. **N.C. Blessin:** None.

P02.07 CHARACTERIZATION OF THE TUMOR IMMUNE MICROENVIRONMENT OF PEDIATRIC POSTERIOR FOSSA A EPENDYMOMAS

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10.1136/jitc-2021-ITOC.19

Background Ependymoma is the third most common brain tumor in children. At the moment, surgery and radiotherapy are the only effective treatments that can be offered, and despite this, a significant part of the patients relapse with no therapeutic salvage options. Therefore, new treatment modalities are needed. To develop immunotherapies for these children, knowledge of the tumor microenvironment is crucial. The current study aims to unravel the tumor immune microenvironment (TIME) of pediatric posterior fossa A (PFA) ependymomas.

Materials and Methods We used bulk RNA sequencing data of 22 pediatric ependymomas. We defined two groups, hereafter called PFA immune+ (PFAI+) and PFAI-, based on the RNA expression levels of the NanoString panel of Human PanCancer Immune Profiling genes. We performed gene set enrichment analysis and deconvoluted the bulk RNA samples with ependymoma-specific single-cell RNA sequencing datasets. To validate our findings on a protein level, we applied immunohistochemistry with antibodies recognizing tumor-infiltrating lymphocytes, tumor-associated macrophages and microglia.

Results Unsupervised hierarchical clustering of RNA expression of immune-related genes revealed two distinct PFA groups. Differential gene expression analysis showed that PFAI+ have a significantly higher expression of genes associated with immune functions, such as CD3E, CCR2, GZMA, CXCL9 and TRBC2. Accordingly, gene set enrichment analysis demonstrated that several immune pathways, including T-cell signalling, interferon-gamma response and TNFα signalling are enriched in PFAI+ ependymomas. RNA expression of immune checkpoints was also higher in PFAI + tumors, indicating that these tumors might be more responsive to combinational therapies including immune checkpoint inhibitors. While immunohistochemistry showed low amounts of infiltrating CD3+, CD8+ and CD20+ cells, high numbers of CD163+ and HLA-DRA+ cells were detected. These cells were mainly located in regions of tumor necrosis. Increased amounts of CD4+ and CD8+ lymphocytes were present in PFAI+ tumors compared to PFAI- tumors. Deconvolution of the bulk RNA samples based on single-cell RNA sequencing data revealed an enrichment of myeloid cell populations, especially microglia and macrophages. Furthermore, PFAI+ tumors were found to contain significantly higher relative proportions of T-cells compared to PFAI- tumors (median of 3.76% for PFAI+ compared to 0.03% for PFAI-).

Conclusions We suggest that pediatric posterior fossa A ependymomas can be divided into two groups based on the expression of immune-related genes, in which PFAI+ ependymomas are characterized by higher RNA expression levels of these genes and greater amounts of tumor-infiltrating immune cells. Several techniques showed an enrichment of T-lymphocytes in the PFAI+ ependymomas relative to the PFAI- ependymomas.

Disclosure Information J. Lammers: None. **F. Calkoen:** None. **M. Kranendonk:** None. **A. Federico:** None. **M. Kool:** None. **L. Kester:** None. **J. van der Lugt:** None.

P02.08 THE ROLE OF FOXP3+ REGULATORY T CELLS AND IDO+ IMMUNE AND TUMOR CELLS IN MALIGNANT MELANOMA – AN IMMUNOHISTOCHEMICAL STUDY

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10.1136/jitc-2021-ITOC.20

Background Although Malignant Cutaneous Melanoma (CM) is a highly immunogenic cancer, it can evade the immune

system by forming an immunosuppressive tumor microenvironment (TME). FoxP3+ Regulatory T cells (Tregs) and indoleamine-2,3-dioxygenase (IDO) are a part of the immunosuppressive TME in CM. In previous studies, IDO expression correlates with poor prognosis and greater Breslow's depth, but results concerning the role of FoxP3+ Tregs in CM have been controversial. Furthermore, the correlation between IDO and Tregs has not been substantially studied in CM, although IDO is known to be an important regulator of Tregs activity. To develop new therapeutic strategies, it is important to understand the role of immunosuppressive factors in CM.

Materials and Methods We investigated the associations of FoxP3+ Tregs, IDO+ tumor cells and IDO+ stromal immune cells with tumor stage, prognostic factors, and survival in CM. FoxP3 and IDO were immunohistochemically stained from 29 benign and 29 dysplastic nevi, 18 in situ - melanomas, 48 superficial and 62 deep melanomas and 67 lymph node metastases of CM. The number of FoxP3+ Tregs and IDO+ stromal immune cells was analysed quantitatively and the coverage and intensity of IDO+ tumor cells was evaluated semiquantitatively. Tumors were divided into IDO-negative and IDO-positive, containing less or more than 1% IDO+ melanoma cells of all tumor cells, respectively. P values equal to or less than 0.05 were considered statistically significant.

Results IDO+ stromal immune cells and FoxP3+ Tregs mainly accumulated in the areas with lymphocyte infiltration and thus resided mostly in the perilesional stroma. The number of FoxP3+ Tregs and IDO+ stromal immune cells were significantly higher in malignant melanomas compared with benign lesions. The increased expression of IDO in melanoma cells was associated with poor prognostic factors, such as recurrence, nodular growth pattern and increased mitotic count. Furthermore, the expression of IDO in melanoma cells was associated with reduced recurrence-free survival. We further showed that IDO-positive tumors contained significantly higher amounts of FoxP3+ Tregs and IDO+ stromal immune cells than IDO-negative tumors. However, the correlation between FoxP3+ Treg and IDO+ stromal immune cell counts was rather weak.

Conclusions Our results indicate that IDO expression is intimately involved in creating a TME conducive to tumor growth in CM. Thus, targeting IDO enzymatic pathway might be a worth of further studies in CM. Furthermore, we show that FoxP3+ Tregs appear to contribute to the immunosuppressive TME in CM, but their role may not be that critical to melanoma progression. The positive association of FoxP3+ Tregs with IDO+ melanoma cells, but not with IDO + stromal immune cells, indicates a complex interaction between IDO and Tregs in CM, which demands further studies. Support: Sigrid Juselius Foundation (S.P.-S.), Academy of Finland (S.P.-S.), The Paavo Koistinen Foundation (S.S.), Emil Aaltonen Foundation (S.S.) and North-Savo Cultural Foundation (S.S.).

Disclosure Information S. Salmi: None. **A. Lin:** None. **B. Hirschovits-Gerz:** None. **M. Valkonen:** None. **N. Aaltonen:** None. **R. Sironen:** None. **H. Siiskonen:** None. **S. Pasonen-Seppänen:** None.

P02.09 HETEROMERIZATION OF UPA AND PAI-1 ENFORCES PRO-TUMORIGENIC NEUTROPHIL TRAFFICKING TO MALIGNANT TUMORS IN BREAST CANCER *VIA* VLDLR-DEPENDENT β2 INTEGRIN CLUSTERING

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10.1136/jitc-2021-ITOC.21

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.

Material 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 and Discussion 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 activates peritumoral macrophages (VLDL receptor- and ERK/MAPK-pathway). This promotes neutrophil trafficking to cancerous lesions (enhanced β2 integrin activation and clustering) and primes these immune cells towards a pro-tumorigenic phenotype (elevated neutrophil elastase expression), 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 Here, we identified an already therapeutically targetable interplay between hemostasis and innate immunity that drives advanced stages of breast cancer as well as characterized the underlying mechanisms of this process. 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. *This study was*

supported by Deutsche Forschungsgemeinschaft (DFG), Sonderforschungsbereich (SFB) 914

Disclosure Information B. Uhl: None. **L. Mittmann:** None. **J. Dominik:** None. **R. Hennel:** None. **B. Smiljanov:** None. **F. Haring:** None. **J. Schaubächer:** None. **C. Braun:** None. **L. Padovan:** None. **R. Pick:** None. **M. Canis:** None. **C. Schulz:** None. **M. Mack:** None. **E. Gutjahr:** None. **P. Sinn:** None. **J. Heil:** None. **K. Steiger:** None. **S.M. Kanse:** None. **W. Weichert:** None. **M. Sperandio:** None. **K. Lauber:** None. **F. Krombach:** None. **C. Reichel:** None.

P02.10 FOCUSCOPE: A SINGLE CELL, MULTI-OMICS SOLUTION TO SIMULTANEOUSLY ANALYZE TUMOR VARIANTS AND MICROENVIRONMENT

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10.1136/jitc-2021-ITOC.22

Recent advances of high-throughput single cell sequencing technologies have greatly improved our understanding of the complex biological systems. Heterogeneous samples such as tumor tissues commonly harbor cancer cell-specific genetic variants and gene expression profiles, both of which have been shown to be related to the mechanisms of disease development, progression, and responses to treatment. Furthermore, stromal and immune cells within tumor microenvironment interact with cancer cells to play important roles in tumor responses to systematic therapy such as immunotherapy or cell therapy. However, most current high-throughput single cell sequencing methods detect only gene expression levels or epigenetics events such as chromatin conformation. The information on important genetic variants including mutation or fusion is not captured. To better understand the mechanisms of tumor responses to systematic therapy, it is essential to decipher the connection between genotype and gene expression patterns of both tumor cells and cells in the tumor microenvironment. We developed FocuSCOPE, a high-throughput multi-omics sequencing solution that can detect both genetic variants and transcriptome from same single cells. FocuSCOPE has been used to successfully perform single cell analysis of both gene expression profiles and point mutations, fusion genes, or intracellular viral sequences from thousands of cells simultaneously, delivering comprehensive insights of tumor and immune cells in tumor microenvironment at single cell resolution.

Disclosure Information Y. Arjmand Abbassi: None. **N. Fang:** None. **W. Zhu:** None. **Y. Zhou:** None. **Y. Chen:** None. **U. Deutsch:** None.

P02.11 TREM1 AGONIST PY159 PROMOTES MYELOID CELL REPROGRAMMING AND UNLEASHES ANTI-TUMOR IMMUNITY

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10.1136/jitc-2021-ITOC.23

Background Tumor-associated myeloid cells can impede productive anti-tumor immunity. One strategy for targeting immunosuppression is myeloid reprogramming, which drives immunosuppressive myeloid cells to acquire an immunostimulatory phenotype. Triggering receptor expressed on myeloid cells-1 (TREM1) is an immunoglobulin superfamily cell surface receptor expressed on neutrophils and subsets of monocytes and tissue macrophages. TREM1 associates with DAP12 adaptor and induces proinflammatory signaling, amplifies innate immune responses, and is implicated in the development of acute and chronic inflammatory diseases. TREM1 is also enriched in tumors, specifically on tumor-associated myeloid cells. To investigate the potential of TREM1 modulation as an anti-cancer therapeutic strategy, we developed PY159, an afucosylated humanized anti-TREM1 monoclonal antibody, and characterized it in the pre-clinical assays described below.

Materials and Methods An FcγR binding ELISA and a Jurkat TREM1/DAP12 NFAT-luciferase reporter cell line were used to assess PY159 binding to human FcγRs and TREM1 signaling, respectively. PY159 responses in human whole blood *in vitro* were evaluated by flow cytometry, transcriptional analysis of sorted leukocyte subsets, and measurement of secreted cytokines/chemokines by MSD. A Transwell system was used to evaluate PY159 effects on neutrophil chemotaxis. TREM1 expression in human tumors was validated by scRNAseq, immunohistochemistry, and flow cytometry. Anti-tumor efficacy of a surrogate anti-mouse TREM1 antibody, PY159m, was evaluated using syngeneic mouse tumor models, either as a single agent or in combination with anti-PD-1.

Results PY159 afucosylation increased its binding affinity for FcγR and its ability to activate TREM1/DAP12 signaling. In human blood assays, PY159 treatment did not induce depletion of TREM1-expressing cells. Rather, it upregulated monocyte activation markers, promoted neutrophil chemotaxis, and induced proinflammatory cytokines and chemokines, which was dependent on PY159 afucosylation. In human tumors, TREM1 was detected on tumor-associated neutrophils, tumor-associated macrophages, and monocytic myeloid-derived suppressive cells. PY159 induced proinflammatory cytokines and chemokines in dissociated human tumors *in vitro*, demonstrating that PY159 can reprogram tumor-associated myeloid cells. A surrogate anti-mouse TREM1 antibody, PY159m, exhibited anti-tumor efficacy in several syngeneic mouse tumor models, both as single-agent and in combination with anti-PD-1.

Conclusions These results show that PY159 is a TREM1 agonist that reprograms myeloid cells and unleashes anti-tumor immunity. PY159 safety and efficacy are currently being evaluated in first-in-human clinical trial (NCT04682431) involving patients resistant and refractory to standard of care therapies.

Disclosure Information V. Juric: A. Employment (full or part-time); Significant; Pionyr Immunotherapeutics Inc. **E. Mayes:** A. Employment (full or part-time); Significant; Pionyr Immunotherapeutics Inc. **M. Binnewies:** A. Employment (full or part-time); Significant; Pionyr Immunotherapeutics Inc. **P. Canaday:** A. Employment (full or part-time); Significant; Pionyr Immunotherapeutics Inc. **T. Lee:** A. Employment (full or part-time); Significant; Pionyr Immunotherapeutics Inc. **S. Dash:** A. Employment (full or part-time); Significant; Pionyr Immunotherapeutics Inc. **J.L. Pollack:** A. Employment (full or part-time); Significant;

Pionyr Immunotherapeutics Inc. **J. Rudolph:** A. Employment (full or part-time); Significant; Pionyr Immunotherapeutics Inc. **V. Huang:** A. Employment (full or part-time); Significant; Pionyr Immunotherapeutics Inc. **X. Du:** A. Employment (full or part-time); Significant; Pionyr Immunotherapeutics Inc. **N. Jahchan:** A. Employment (full or part-time); Significant; Pionyr Immunotherapeutics Inc. **A.J. Ramoth:** A. Employment (full or part-time); Significant; Pionyr Immunotherapeutics Inc. **S. Mankikar:** A. Employment (full or part-time); Significant; Pionyr Immunotherapeutics Inc. **M. Norng:** A. Employment (full or part-time); Significant; Pionyr Immunotherapeutics Inc. **C. Santamaria:** A. Employment (full or part-time); Significant; Pionyr Immunotherapeutics Inc. **K.P. Baker:** A. Employment (full or part-time); Significant; Pionyr Immunotherapeutics Inc. **L. Liang:** A. Employment (full or part-time); Significant; Pionyr Immunotherapeutics Inc.

P03 Vaccine Therapy

P03.01 HIGH IMMUNOGENIC VLP-BASED VACCINES ELICIT NEW T CELL SPECIFICITIES AGAINST MELANOMA NEOANTIGENS IN MICE

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10.1136/jitc-2021-ITOC.24

Background Neoantigens' (neoAg) identification, which determines T-cell responses against tumors, has fostered the development of personalized vaccines with promising results. While the ranking of the most immunogenic neoAg can be addressed using predictive techniques, their formulation as vaccines needs to be improved. To maximize their therapeutic potential, optimal neoAg-based vaccines should be manufactured in a superb delivery platform that enhances robust new immune responses, able to bypass thymic tolerance and the humoral immunosuppressive microenvironment. These novel T cell responses generated at the periphery will not be exhausted, opposite to TILs. We aim to develop a highly immunogenic vaccine platform, based on engineered HIV-derived Virus-Like Particles (VLP) expressing approximately 2500 copies of each selected neoAg. We tested different neoAgs loaded VLPs (neoVLP) in a melanoma mouse model to evaluate their capability to generate new immunogenic specificities.

Material and Methods Specific non-synonymous mutations from B16F10 cells were identified, selected and used to generate a list of prioritized peptides. NeoAgs were classified as: Tier1, acquiring a mutation that creates an anchor residue to the MHC-I, not present in the WT peptide; Tier2, acquiring a mutation in a position that largely impacts contact with the TCR respect to WT; and Tier3, acquiring a mutation in the TCR contact region but inducing a less drastic change than in Tier2. Frame shift (FS) mutations, expected to be highly immunogenic, were also included.Thirteen to fifteen selected neoAgs from each group were loaded on highly immunogenic neoVLPs. Their immunogenicity was evaluated in C57bl/6 mice by immunization with a neoVLP-coding plasmid DNA (prime) and purified neoVLPs as soluble particles (boost). Splenocytes were used to evaluate neoAg-specific T cell responses.

Results We have successfully generated and purified neoVLPs, exposing neoAgs from all groups by transient transfection of Expi293 cells. Protein integrity and VLP morphology were confirmed by western blot and cryo-EM. When used for immunization assays, neoVLPs, containing neoAgs from Tier2, Tier3 and FS groups, were capable of generating humoral responses against viral proteins and T cell responses against neoAgs present in the neoVLP. B16F10 inoculated animals, but not vaccinated, did not develop detectable T cell responses against neoAgs present in any tested neoVLP, suggesting that the vaccination with neoVLPs promoted new specificities against selected neoAgs that might contribute to tumor control and eradication.

Conclusion Our data show that the neoVLPs promote the generation of new antitumor-specific immune responses against selected neoepitopes, suggesting that neoVLPs vaccination could be an alternative to current therapeutic vaccine approaches and a promising candidate for future personalized immunotherapy.

Disclosure Information C. Aguilar-Gurrieri: None. **A. Barajas:** None. **A. Pons-Grifols:** None. **I. Varela:** None. **P. Amengual-Rigo:** None. **R. Farriol:** None. **M. Vazquez:** None. **R. Lepore:** None. **C. Anjos-Souza:** None. **J. Blanco-Heredia:** None. **L. de Mattos-Arruda:** None. **V. Guallar:** None. **A. Valencia:** None. **B. Clotet:** F. Consultant/Advisory Board; Significant; Albajuna Therapeutics. **J. Carrillo:** Other; Significant; Albajuna Therapeutics. **J. Blanco:** F. Consultant/Advisory Board; Significant; Albajuna Therapeutics.

P03.02 PROTEIN-BASED CANCER VACCINE COMBINED WITH AN ONCOLYTIC VACCINE PROMOTES POTENT ANTITUMOR IMMUNITY

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10.1136/jitc-2021-ITOC.25

Background KISIMA^{TM} platform allows the development of protein-based cancer vaccines able to induce a potent, tumor-specific CD8 and CD4 T cells response. While the cell penetrating peptide and peptide agonist for Toll like receptor (TLR)-2 and TLR-4 confer, respectively, the cell delivery and self-adjuvanticity properties, the multiantigenic domain allows the targeting of different cancer antigens, resulting in antitumoral efficacy in different murine models. Oncolytic viruses exert their therapeutic effects by a prolonged oncolytic action and the associated intratumoral inflammation as well as general immune activation. Arming oncolytic virus with tumor associated antigens can additionally enhance the tumor-specific T cell portion and therefore positively affect the balance of antitumor versus antiviral immune responses. The protein vaccine KISIMA^{TM} and the recombinant oncolytic virus VSV-GP-TAA (vesicular stomatitis virus pseudotyped with LCMV GP expressing tumor-associated antigens) are both promising vaccine candidates that offer a new

cancer vaccination opportunity when combined in heterologous prime-boost regimen.

Materials and Methods Mice were vaccinated with subcutaneous (s.c.) injection of KISIMA-TAA vaccine and/or with intravenous injection of VSV-GP-TAA in different settings. Immunogenicity was assessed by measuring the peripheral antigen-specific response. Anti-tumoral efficacy as well as in depth monitoring of TILs and tumor microenvironment modulation were assessed following therapeutic vaccination in different tumor models. Additionally, transcriptome and immunohistochemistry analyses of the TC-1 tumor have been performed. Combination of heterologous prime-boost with checkpoint blockade PD-1 therapy has been assessed.

Results Priming with KISIMA-TAA followed by VSV-GP-TAA boost induced a large pool of polyfunctional and persistent antigen-specific cytotoxic T cells in the periphery as well as within the tumor in several tumor models. Frequencies of antigen specific T cells are significantly higher than the respective homologous vaccinations. Additionally, transcriptome analysis of a cold tumor model revealed profound changes in the tumor microenvironment upon heterologous vaccination, including a strong upregulation of gene signatures of several pro-inflammatory cytokines and chemokines required for anti-tumor immunity along with dendritic and T cell trafficking and activation. This was corroborated by flow-cytometric analysis of tumor-infiltrating leukocytes showing massive $CD8^+$ and $CD4^+$ T cell infiltration as well as repolarization of M2-like macrophages towards M1-phenotype. The presence of the $CD8^+$ T cells within the tumor core was confirmed by immunohistochemistry analysis. Moreover, combining heterologous vaccination with checkpoint blockade further improved its therapeutic efficacy and the number of long-term survivors.

Conclusions The KISIMA/VSV-GP heterologous prime-boost approach holds great promise for patients with primary or acquired resistance to checkpoint blockade due to its ability to induce tumor-specific T cell, improve T cell infiltration and increase tumor inflammation, even in tumors with limited permissivity for the oncolytic virus.

Disclosure Information E. Belnoue: A. Employment (full or part-time); Significant; AMAL Therapeutics SA. **K. Das:** None. **M. Rossi:** A. Employment (full or part-time); Significant; AMAL Therapeutics SA. **T. Hofer:** None. **S. Danklmaier:** None. **T. Nolden:** A. Employment (full or part-time); Significant; Viratherapeutics GmbH. **L. Schreiber:** None. **K. Angerer:** None. **J. Kimpel:** None. **S. Hoegler:** None. **L. Kenner:** None. **D. von Laer:** None. **K. Elbers:** A. Employment (full or part-time); Significant; Viratherapeutics GmbH. **G. Wollmann:** None. **M. Derouazi:** A. Employment (full or part-time); Significant; AMAL Therapeutics SA.

P03.03 NOVEL MACHINE-LEARNING TOOLS IMPROVE COST-EFFECTIVE DEVELOPMENT OF PERSONALISED IMMUNOTHERAPIES: LOWERING FALSE POSITIVE RATES IN THE SEARCH FOR ACTIONABLE IMMUNOGENIC NEOANTIGENS

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10.1136/jitc-2021-ITOC.26

Background Personalised immunotherapy approaches rely on the ability of tumour-derived neoantigens to elicit a T-cell immune reaction able to recognise and kill the tumour cells expressing them. Clinical attempts to leverage the power of neoantigens have however yielded mixed results. This can mostly be attributed to the difficulty of finding truly immunogenic peptides from the set of novel peptides generated by mutations in a given cancer patient. In silico approaches can help alleviate this heavy cost by reducing the neoantigen search space, prioritising epitopes based on various parameters such as epitope expression or MHC binding likelihood. Here we present a suite of tools aimed at further assisting clinicians in selecting the most actionable peptides from a set of potential candidates.

Materials and Methods We developed neoMS, a neural network algorithm able to predict epitope presentation at the cell surface with unparalleled performance. Furthermore, the neoIM algorithm is able to discriminate, in an HLA-agnostic fashion, which of the presented peptides will elicit a T-cell immune reaction. This first-in-class algorithm is a random forest classifier specifically trained to classify short peptides of length 9-11 amino acids as immunogenic or non-immunogenic.

Results The neoMS model achieves up to 0.61 precision at recall 0.4 on its test set, vastly outperforming the current industry standards. In addition, due to his sequence-based comparison method, neoMS exhibits extrapolation capabilities, achieving non-zero predictive power when evaluated on ground truth ligandome data derived from an HLA allele completely absent from the training set. In some cancer indications we showed, moreover, that the neoMS-predicted rate of neoantigen presentation can be used in combination with tumour mutational burden as a high-specificity predictor of response to immune checkpoint inhibitor treatment. neoIM vastly outperforms the currently available methods and can predict peptide immunogenicity with high accuracy (AUC=0.88). Interestingly, neoIM confirmed ELISPOT data obtained by Dillon et al. (2017) showing a response in 4 out of 11 breast cancer patients to a vaccine consisting of 9 MHC class-I restricted breast cancer-associated peptides. The 2 antigens that resulted in a CD8+ T-cell specific response were predicted by neoIM as the highest scoring showing its potential in finding the truly immunogenic neoantigens.

Conclusions Taken together, these tools decrease false positive rates significantly as they enable improved identification of immunogenic peptides and the predictions correlate with intensity of immune response and clinical benefits. As such, these tools represent a cost-efficient preliminary step in the search for actionable, immunogenic neoantigens.

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Disclosure Information C. Bogaert: None. **L. Van Oudenhove:** None. **L. Pfitzer:** None. **N. Mill:** None. **B. Fant:** None.

P03.04 NOVEL ALGORITHM DISCOVERS UP TO 35 PERCENT MORE EPITOPES TRANSLATED FROM NON-CODING REGIONS IN COLD TUMOURS

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10.1136/jitc-2021-ITOC.27

Background Currently, most neoantigen pipelines often focus on the detection of neoantigens derived from mutations in the coding regions of the genome. However, in some cancer indications, the number of mutations detectable in tumours can be very low (low tumour mutational burden). This limits the number of actionable neoantigens and results in so-called 'cold' tumours. In these cases, non-canonical neoantigens resulting from alterations in non-coding regions of the human genome could represent a high potential alternative for treatment.

Indeed, recent research has revealed that previously presumed non-coding regions of the human genome, such as long non-coding RNAs (lncRNAs), can contain translatable small open reading frames (smORFs) generating micropeptides. Some of these micropeptides have already been shown to be involved in cancer development, but these small peptides could also represent a high potential source of non-canonical neoantigens for personalised therapy.

Materials and Methods Here, we present smORFin, a machine learning algorithm specifically trained to identify smORFs in transcripts and to assess their coding potential. While most tools are focused on longer sequences, smORFin is specifically developed to target small ORFs (<303 nucleotides). Furthermore, smORFin also accounts for smORFs with alternative initiation codons, thereby improving its sensitivity for the detection of novel unannotated smORFs.

In addition, the impact of mutations in allegedly non-coding regions of tumour genomes and its influence on the neoantigen repertoire, was evaluated through integration of smORFin in a neoantigen identification pipeline targeting lncRNA-derived mutated epitopes; lncRNeos.

Results The smORFin model reaches a precision of 0.98 and an accuracy of 0.95 on its testing dataset. Using this new prediction tool, a library of human smORFs was assembled, the so-called smORFeome. This library of smORFs, and their associated proteins, was evaluated as a reference for spectrum to peptide matching in mass spectrometry data (MS) analysis. Indeed, the evaluation of seven MS samples revealed and validated the presence of smORFeome-related micropeptides and HLA-I associated epitopes originating from smORFs.

Furthermore, it was observed that lncRNA-derived epitopes only represent a minor fraction of the total neoantigen load. Strikingly, when only focusing on tumours with a low neoantigen load, lncRNeos represented up to 27% of the total neoantigen load. This indicates that for tumours with a low TMB, and therefore with a low neoantigen load, lncRNeos allows to significantly expand the neoantigen repertoire. Biological in vivo/in vitro validation remains necessary to assess the existence, presentation, and actionability of lncRNeos.

Conclusions A novel random forest-based algorithm was developed to address the need for reliable identification of lncRNA-born smORFs. Furthermore, the integration of this prediction algorithm in a neoantigen pipeline allowed the identification of lncRNA-derived neoantigens and marks them as a potential novel source for personalised immunotherapy.

Disclosure Information C. Bogaert: None. **L. Van Oudenhove:** None. **B. Fant:** None.

P03.06 DECIPHERING VACCINE-INDUCED NEOEPITOPE-SPECIFIC T CELL RESPONSES IN A PATIENT WITH H3K27M-MUTANT MIDLINE GLIOMA WHO GRADE 4

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10.1136/jitc-2021-ITOC.28

Background K27M-mutant histone-3 (H3K27M) defines a clinically and molecularly distinct entity of diffuse midline gliomas WHO grade 4 with an unfavorable prognosis. From an immunological perspective, H3K27M constitutes a cancer neoepitope: in a syngeneic MHC-humanized mouse model, an H3K27M-specific long peptide vaccine induced mutation-specific T cells responses capable of inhibiting growth of H3K27M-expressing tumors.

Materials and Methods Here, we describe clinical response of a patient diagnosed with H3K27M-mutant midline gliomas to H3K27M-specific peptide vaccination and exploit vaccine-induced T cell phenotypes.

Results Repeated peptide vaccinations were well tolerated and resulted in long-term response after pseudoprogression. Longitudinal immune monitoring showed induction of H3K27M-specific CD4-driven T cell responses in the peripheral blood. Within the cerebrospinal fluid, expansion of HLA-specific vaccine-induced T cell receptor (TCR) clones was observed and associated with distinct HLA types.

Conclusions Identification of vaccine-induced TCR clones within the peripheral blood and CSF of patients with H3K27M-mutant midline glioma may be used to prioritize TCRs for adoptive T cell therapy.

Disclosure Information K. Lindner: None. **K. Kromer:** None. **K. Jähne:** None. **L. Bunse:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Patent: US20180155403A1 (Histone Anti-Cancer Vaccines). **C. Tan:** None. **I. Poschke:** None. **E. Green:** None. **J.M. Lindner:** None. **M. Platten:** C. Other Research Support (supplies, equipment, receipt of drugs or other in-kind support); Modest; Roche. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Patent: US20180155403A1 (Histone Anti-Cancer Vaccines). **K. Sahm:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Patent: US20180155403A1 (Histone Anti-Cancer Vaccines).

P03.07 ANALYSIS OF SCRNASEQ FROM THE HUMAN THYMUS NOMINATES GENES POTENTIALLY MISSING FROM CENTRAL TOLERANCE OF CYTOTOXIC T CELLS

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10.1136/jitc-2021-ITOC.29

Background During thymic development, cytotoxic T cells that can bind to and attack self antigens undergo negative selection thus preventing damage to the self tissues. The sparse medullar thymic epithelial cells (mTECs) present in the thymus are responsible for presenting self antigens to T cells so that they

can trigger apoptosis or differentiation into non-cytotoxic lineages if they bind too strongly.

Materials and Methods Understanding gene expression in mTECs is essential for understanding the shape of the human T cell receptor repertoire, which is key for current and emerging cancer immunotherapies. Recent availability of human thymus single cell RNAseq (scRNAseq) data provides an extremely high-resolution view into the pattern of expression within this critical cell type. To determine which epitopes have had to opportunity to be presented during T cell negative selection, we analyzed the human thymus scRNAseq dataset to establish which genes are expressed in mTECs and therefore subject to central tolerance.

Results The coverage of the whole transcriptome of a particular cell is generally sparse. It is therefore difficult to understand basic features of individual cells or cell types such as how many genes are expressed. We used cell- and read-level subsampling to estimate whether a sufficient number of cells and reads had been captured to support categorizing a gene as non-expressed in mTECs. We also examined the expression of the genes not expressed in mTECs in other healthy tissues, and found their expression was almost exclusively restricted to the testis (an immune-privileged site) and the liver (a site of peripheral tolerance)

Conclusions Altogether, these analyses establish a strategy for determining if a data set has sufficient depth to estimate the total number of genes expressed and secondly define a key list of genes that are not expressed during central tolerization of T cells, which represent a compelling list of possible cancer immunotherapy targets.

Disclosure Information L. Blumenberg: A. Employment (full or part-time); Significant; Regeneron Pharmaceuticals. **G. Atwal:** A. Employment (full or part-time); Significant; Regeneron Pharmaceuticals. **A. Dhanik:** A. Employment (full or part-time); Significant; Regeneron Pharmaceuticals.

P04 Precision Medicine Meets Immunotherapy (Immuno-Monitoring)

P04.01 IMMUNOMONITORING OF CD19. CAR T-CELLS IN LARGE B-CELL LYMPHOMA- A TWO-CENTER EXPERIENCE

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10.1136/jitc-2021-ITOC.30

Background CD19. CAR T-cells for the treatment of relapsed and refractory (r/r) Diffuse Large B-Cell Lymphoma (DLBCL) demonstrated complete responses in 40%-58% of the patients. Recently, others could associate high tumor volume and low CAR T-cell expansion *in vivo* with poor outcome. We hypothesize, that the expansion and immunphenotype of (CAR) T cells *in vivo* determine treatment response and depend on patient- and disease associated factors.

Materials and Methods Patients with r/r DLBCL (n=34) were treated with either Axi-cel or Tisa-cel at the University Hospitals of Erlangen and Munich (LMU). The CAR T-cell product and peripheral blood were collected on day 0, 4, 7, 14, 30, 60 and 90 post transfusion. CAR T-cells were detected through flow cytometry utilizing a two step-staining with a biotinylated CD19 protein. Effector:Target (E:T) Ratios were estimated as absolute peak expansion of CAR T-cells (/ul) per tumorvolume (cm^3). Responder (R, complete or partial remission) were compared to Non-Responder (NR, stable or progressive disease) according to response assessment with PET-CT three months after transfusion.

Results CAR T-cell expansion peaked between day 7 and day 14 after transfusion with a greater expansion of $CD8^+$compared to $CD8^-$ CAR T-cells on day 14 (59.27% vs 37.42%, p=0.021). The ratio of $CD8^+$ and $CD8^-$ CAR T-cells did not differ between R and NR, however R exhibited higher E:T ratios of $CD3^+$ CAR T-cells compared to NR (20.94 vs 12.81, p=0.015) and an increased E:T ratio of $CD8^+$ CAR T-cells correlated with better progression-free survival (p=0.033). Interestingly, high CRP and ferritin levels at baseline were inversely associated with the E:T ratio (p=0.048 and p=0.017). $CD3^+$ CAR T-cells of R showed earlier peak expression of PD-1 than NR (day 7 vs day 21). Further, peak expansion of $CD3^+$ CAR T-cells correlated with higher PD-1 expression in R but not in NR (p=0.003 vs p=0.12). In addition, R revealed an increased relative frequency of effector memory differentiated $CD3^+$ CAR T-cells ($CCR7^-CD45RA^-$, p=0.02), whereas CAR T-cells in NR showed an increased relative frequency of a naïve phenotype ($CCR7^+CD45RA^+$, p=0.001) on day 7 post infusion.

Conclusions Flow-based immunomonitoring with longitudinal characterization of CAR T-cells demonstrated a correlation of the E:T ratio with treatment response and survival. Increased inflammatory conditions at baseline correlated with diminished E:T ratios. Notably, in R CAR peak expansion was positively associated with higher PD-1 expression suggestive for superior CAR T-cell activation. In addition, greater memory differentiation was associated with efficacy during the time of peak expansion. Multiparameter analysis with other clinical covariates will show, whether CAR T-cell expansion and immunphenotypes can predict patient outcome.

Disclosure Information V. Blumenberg: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Novartis, Gilead Sciences, Janssen, BMS/Celgene. **S. Völkl:** None. **G. Busch:** None. **S. Baumann:** None. **M. Winkelmann:** None. **B. Tast:** None. **D. Nixdorf:** None. **G. Hänel:** None. **L. Frölich:** None. **C. Schmidt:** None. **R. Jitschin:** None. **F. Vettermann:** None. **W. Kunz:** None. **D. Mougiakakos:** None. **M. von Bergwelt:** None. **V. Bücklein:** None. **A. Mackensen:** None. **M. Subklewe:** None.

P04.02 DIVERSITY OF CD4+ BLOOD T-CELL CLONALITY PREDICTS LONGER SURVIVAL WITH CTLA4 OR PD-1 CHECKPOINT INHIBITION IN ADVANCED MELANOMA

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10.1136/jitc-2021-ITOC.31

Background T cells play a central role in tumor immunity. In principle, T cell requires antigen recognition by T-cell receptor (TCR) to gain effector function. Antigen-driven activation leads to clonal T-cell expansion with generation of progeny cells that all express the same chronotypic TCR. This makes TCR analysis a useful tool to comprehensively and individually understand antigen-specific T-cell responses. Indeed, we previously showed that the TCR repertoires of $CD8^+$ T cells but not $CD4^+$ T cells are restricted with many clones in the blood of psoriasis patients. Together with the strong genetic association to HLA-C*06:02 causing an autoimmune $CD8^+$ T-cell response against melanocytes in psoriasis, our results from TCR analyses clearly indicate an autoimmune pathogenesis of psoriasis.
Patients and Methods Here, we utilize our expertise to understand how anti-tumor T-cell responses affect clinical responses and immune-related adverse events (irAEs) in therapeutic checkpoint inhibitions. We analyzed melanoma patients upon the therapeutic blockade of cytotoxic T-lymphocyte-associated protein 4 (CTLA4) or programmed cell death 1 (PD-1) using TCR Vβ-gene spectratyping.
Results Surprisingly, we observed variable levels of restriction in $CD4^+$ and extensive restrictions in $CD8^+$ T-cell repertoires in the blood of melanoma patients compared to healthy controls. This indicates the presence of a substantial numbers of $CD4^+$ and $CD8^+$ T-cell clones in the blood prior to the initiation of immunotherapy. The clones detected in the blood were enriched in tumor-infiltrating lymphocytes (TILs). This suggests that melanoma-reactive T-cell clones circulate more frequently in melanoma patients, although it is generally assumed that tumor-specific T-cell clones are only detectable in TILs. Greater diversification particularly in $CD4^+$ blood T-cell clones before immunotherapy correlated with long-term survival after CTLA4 or PD-1 inhibition. In patients who developed severe immune-related adverse events (irAEs) during CTLA4 blockade, we detected newly expanded blood T-cell clones, suggesting that newly emerged T-cell responses contributed to these irAEs.
Conclusions Our data demonstrate that the diversity of T-cell clones in the circulation may reflect the anti-melanoma responses. This study provides a rationale for predicting clinical responses to checkpoint inhibitors using patient's blood, and also emphasizes importance of $CD4^+$ T cell-mediated anti-tumor immunity in melanoma.

Disclosure Information A. Arakawa: None. **S. Vollmer:** None. **J. Tietze:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; BMS. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; BMS, MSD, Novartis, Roche, Almiral. **A. Galinski:** None. **M. V. Heppt:** None. **C. Berking:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Amgen, AstraZeneca, BMS, Incyte, Merck, MSD, Novartis, Pierre Fabre, Regeneron, Roche, Sanofi/Aventis. **J.C. Prinz:** None.

P04.03 EXPRESSION PROFILES OF IMMUNE MARKERS AS PREDICTORS OF SURVIVAL IN SURGICALLY-TREATED NSCLC

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10.1136/jitc-2021-ITOC.32

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. There is now increased awareness about the physiological role of lymph nodes in cancer. We investigated the expression profiles of immune-related markers in matched tumor tissue, affected and unaffected N1 and N2 lymph nodes in patients with NSCLC and their relation to survival.
Materials and Methods Internal hospital databases were screened for surgically-treated NSCLC patients with documented relapse or long-term disease-free survival (defined as 3 years). Data on patients' age, sex, surgery, (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. mRNA was extracted from these tissues and expression profiling of 751 immune-related genes was performed using the PanCancer IO 360 panel by NanoString Technologies. **Results**

A total of 754 NSCLC patients were screened. Of these, 71 patients showed long-term disease-free survival and 80 patients had local or systemic relapse within 3 years after surgery. Expression profiles of immune-related genes in tumor and lymph node immune populations differed between patients with and without 3-year disease-free survival.
Conclusions Expression profiles of immune-related genes differ between patients with and without relapse. Our findings show that differences in expression profiles of immune-related genes in tumor and lymph nodes should be taken into account when assessing patient prognosis.

Disclosure Information L. Sellmer: None. **J. Kovács:** None. **J. Kumbrink:** None. **J. Neumann:** None. **J. Walter:** None. **D. Kauffmann-Guerrero:** None. **C. Schneider:** None. **A. Tufman:** None.

P04.04 PROGRAMMED DEATH-LIGAND 1 POSITRON EMISSION TOMOGRAPHY IMAGING DURING NEOADJUVANT (CHEMO)RADIOTHERAPY IN ESOPHAGEAL AND RECTAL CANCER (PETNEC): A PROSPECTIVE NON-RANDOMIZED OPEN-LABEL SINGLE-CENTER PILOT STUDY

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10.1136/jitc-2021-ITOC.33

Background Immune checkpoint inhibitors (ICI), such as atezolizumab (anti-programmed death-ligand 1; PD-L1), have been proven to be an effective strategy in solid cancers. However, the overall response rate to ICI is currently limited to an intrinsically responsive tumor immune microenvironment (TIME) or depended on an appropriate foregone immune stimulus, such as radiotherapy. The concept of combining radiotherapy with ICI is currently investigated in a variety of solid cancers. However, little data is known on the expression dynamics of immune checkpoint ligands, such as PD-L1, during neoadjuvant chemoradiotherapy (CRT) or short-course preoperative radiotherapy (SCPRT) in human solid malignancies.

Materials and Methods This is a prospective non-randomized open-label single-center investigator-initiated pilot study (NCT no. NCT04564482). Patients with either rectal cancer (RC), oesophageal adenocarcinoma (EAC), gastroesophageal junction (GEJ) cancer or oesophageal squamous cell carcinoma (ESCC), whom are assigned by the routine multidisciplinary tumor board (MDT) to receive a standard neoadjuvant CRT/SCPRT, will be enrolled. Standard neoadjuvant regimens include CRT (50 Gy in 2 Gy fractions over 25 working days + capecitabine 1650 mg/m^2/d PO) or SCPRT (25 Gy in 5 Gy fractions over 5 working days) for RC patients and CRT according to the CROSS protocol (41.4 Gy in 1.8 Gy fractions over 23 working days + carboplatin AUC of 2 mg/ml/min + paclitaxel 50 mg/m2 IV Q1W) for patients with EAC, ESCC or GEJ cancer. Patients will receive a PD-L1 (^{89}Zr-atezolizumab) positron emission tomography (PET) CT (for EAC, ESCC or GEJ cancers) or MRI (for RC) bevor (day 0) and during neoadjuvant treatment (day 10-14).

Results The primary endpoint of this pilot study is the none-invasive assessment of PD-L1 expression dynamics during neoadjuvant CRT/SCPRT by a PD-L1 PET imaging approach. Secondary objectives are the correlation between PD-L1 PET expression dynamics and radiographic as well as pathological therapy response.

Conclusions This is the first in human study, which assesses PD-L1 expression dynamics during different neoadjuvant radiotherapeutic regimens. A detailed understanding of the impact of radiotherapy on PD-L1 expression, monitored by an none-invasive PET imaging approach, allows the application of radiotherapy as part of a novel immunotherapeutic concept.

Disclosure Information J. Laengle: None. **D. Tamandl:** None. **R. Schmid:** None. **J. Widder:** None. **M. Bergmann:** None. **A. Haug:** None.

P05 'Lost in Translation'?

P05.01 ORGANOID-SPECIFIC OPTIMIZATION OF KILLING ASSAYS TO TEST NOVEL IMMUNOTHERAPIES IN A HIGH-THROUGHPUT SYSTEM

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10.1136/jitc-2021-ITOC.34

Background The immunotherapeutic drug dinutuximab, which binds to disialoganglioside (GD2) and activates natural killer (NK) cells, is part of the standard regimen in high-risk neuroblastoma (NB) patients. However, dinutuximab only results in tumor reduction in a subset of patients, and survival rates of high-risk neuroblastoma patients are below 60%. Novel immunotherapies are therefore needed. Current in vitro models lack the ability to study novel immunotherapies with high-throughput screening (HTS). We aimed to optimize NB organoid-lymphocyte cocultures for HTS, and possibly personalized testing, of novel antibody-mediated and cellular immunotherapies.

Materials and Methods Two patient-derived organoids (691B: GD2$^+$MHC-I$^-$ and 691T: GD2$^-$MHC-I$^+$) were transduced with an endogenous luciferase construct to use D-luciferin-induced bioluminescence as readout for cell growth. The growth rate, optimal seeding density and optimal pre-culture time per organoid were determined by density curves, and the number of needed cells was downscaled to facilitate HTS. After pre-culture, the luciferase-transduced organoids were co-cultured with primary PBMCs from healthy donors, PRAME-TCR transduced T cells or CAR-T cells.[1] Several effector:target (E:T) ratios and timepoints were tested to identify the optimal window for read-out of dinutuximab-induced antibody-dependent cytotoxicity (ADCC) and T-cell mediated cytotoxicity. The required number of immune cells per ratio was calculated based on the expansion rate of organoid cells after 48 and 72 hours.

Results The density screens showed an optimal seeding density of 5000-10.000 organoid cells per well, yielding a high luminescence signal while minimizing the number of cells needed. Already at the lowest E:T ratio (1:3), we observed killing of the MHC-I expressing 691T organoid, likely based on allogeneic recognition of the organoids by T cells. The killing efficacy increased with higher E:T ratios and co-culture time. Pre-culturing of organoids for 72 hours before addition of effector cells resulted in formation of larger 3D spheres, which reduced the killing efficacy for all E:T ratios. ADCC effects of dinutuximab were studied in GD2$^+$MHC-I$^-$ 691B organoids. Addition of dinutuximab resulted in 25% increase of killing after 24 hours and reached up to 70% increase after 72 hours for 10:1 and 20:1 E:T ratios. Higher E:T ratios were likely needed because NK cells make up a smaller proportion of PBMCs than T cells. Dinutuximab did not increase killing of the GD2$^-$ organoid, confirming specificity of the antibody. T cell mediated killing was almost 100% for MHC-I$^+$691T organoids after 24 hours of culturing with PRAME-TCR transduced T cells and CAR-T cells at a 1:3 E:T ratio, showing the high anti-tumor cytotoxicity of these cells and potential for HTS at very low E:T ratios.

Conclusions We have developed a robust in vitro bioluminescence-based organoid/lymphocyte co-culture assay with a low cell input, to facilitate high-throughput screening of novel antibody-based or cellular immunotherapies, possibly combined with chemotherapeutic or targeted compounds. In the future this method may be applied for personalized drug screens.

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Disclosure Information F. van den Ham: None. **W.M. Kholosy:** None. **K. Ober:** None. **A.M. Cornel:** None. **S. Nierkens:** None. **J. Anderson:** None. **J.J. Molenaar:** None. **J. Wienke:** None.

P06 Cell Therapy in Solid Tumors

P06.01 αβ-T CELLS ENGINEERED TO EXPRESS γδ-T CELL RECEPTORS CAN KILL NEUROBLASTOMA ORGANOIDS INDEPENDENT OF MHC-I EXPRESSION

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10.1136/jitc-2021-ITOC.35

Background Currently ~50% of patients with the diagnosis of high-risk neuroblastoma will not survive due to relapsing or refractory disease. Recent innovations in immunotherapy for solid tumors are highly promising, but the low MHC-I expression of neuroblastoma represents a major challenge for T cell-mediated immunotherapy. Here, we propose a novel T cell-based immunotherapy approach for neuroblastoma, based on the use of TEG002, αβ-T cells engineered to express a defined γδ-T cell receptor, which are thought to recognize and kill target cells independent of MHC-I. In this pilot project we have tested the potential efficacy of TEG002 therapy as a novel treatment for neuroblastoma, with tumor organoids.

Materials and Methods Effector cells were created from healthy donor peripheral blood T cells. The TEG002 cells were engineered by transducing αβ-T cells with a defined Vγ9Vδ2-T cell receptor. Both the untransduced αβ-T cells and the endogenous Vγ9Vδ2-T cells from the same healthy donor were used as controls in all experiments. Activation and killing of TEG002 was tested in a co-culture setting with neuroblastoma organoids. Supernatant of the co-culture was collected at 24 hours for IFNγ ELISA to measure activation of TEG002. The dynamics of cytotoxicity were analyzed over time from 0 till 72 hours, using the live-cell imaging system IncuCyte from Sartorius®. Killing was quantified using a Caspase3/7 Green dye and the IncuCyte software. Transcriptional profiling of the neuroblastoma organoids was done by RNA sequencing and MHC-I expression of the neuroblastoma organoids was determined by flow cytometry.

Results We showed that 3 out of 6 neuroblastoma organoids could activate TEG002 as measured by IFNγ production. Transcriptional profiling of the neuroblastoma organoids showed that this effect correlates with an increased activity of processes involved in interferon signaling and extracellular matrix organization. Analysis of the dynamics of organoid killing by TEG002 over time confirmed that organoids which induced TEG002 activation were efficiently killed independently of their MHC-I expression. Of note, efficacy of TEG002 treatment was superior to donor-matched untransduced αβ-T cells or endogenous γδ-T cells.

Conclusions We demonstrated that 50% of tested neuroblastoma organoids can effectively activate TEG002 and that killing of the organoids is independent of MHC-I expression. Hence, this pilot study identified TEG002 as a promising novel cellular product for immunotherapy for a subset of neuroblastoma tumors, warranting further investigations into its clinical application.

Disclosure Information J.G.M. Strijker: None. **E. Drent:** A. Employment (full or part-time); Significant; Gadeta BV. **J.J.F. van der Hoek:** None. **R. Pscheid:** A. Employment (full or part-time); Significant; Gadeta BV. **B. Koopmans:** None. **K. Ober:** None. **S.R. van Hooff:** None. **W.M. Kholosy:** None. **C. Coomans:** A. Employment (full or part-time); Significant; Gadeta BV. **A. Bisso:** A. Employment (full or part-time); Significant; Gadeta BV. **M. van Loenen:** A. Employment (full or part-time); Significant; Gadeta BV. **J.J. Molenaar:** None. **J. Wienke:** None.

P06.02 CANCER-SPECIFIC DIFFERENCES OF TERTIARY LYMPHOID STRUCTURES AND CELLULAR RESPONSES AGAINST FREQUENTLY EXPRESSED CANCER TESTIS ANTIGENS

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10.1136/jitc-2021-ITOC.36

Background Tertiary lymphocyte structures (TLS) can be detected in the tumor microenvironment across a wide range of cancer types and are associated with increased patient survival and susceptibility to immune checkpoint inhibition. However, evidence for the functional significance of TLS on humoral and cellular immunity is scarce. In this study, we combine assessment of abundance and spatial distribution of TLS with expression levels of 10 tumor associated antigens (TAAs) and functional analyses of T cell responses to these antigens.

Materials and Methods 52 treatment naïve cancer patients across 5 tumor types (NSCLC, CRC, RCC, HCC and BCA) were included. Presence and localization of TLS was assessed in immunohistochemical stainings (CD20) of whole section slides from FFPE embedded tumor samples. B cell clusters were quantified in the whole tumor region and in two different tumor margins (300 µm, 2000 µm). A panel of 30 cancer testis antigens was selected via GEPIA software (TCGA Database) and their expression in our cohort was determined using NanoString based RNA expression analysis of tumor samples and patient-matched healthy tissue. The 10 peptide pools with the largest cross-cancer overlap were selected based on our NanoString results. 2-color Fluorospot assays (IFN-γ and IL-2) were applied to assess the frequency of tumor-specific T-cell responses in patient PBMCs (triplicates for each TAA).

Results CD20 immunohistochemistry and enumeration of intra- and peritumoral TLS revealed different distribution patterns of TLS/mm^2 with the largest proportion in the 300 µm margin ($p < 0.01$) in most of the cancer types. This effect was particularly observed in patients with non-small cell lung cancer (NSCLC). The 10 tumor antigens CEP55, CT83, GAGE1, IGF2BP3, MAGEA1, MAGEA3/6, PBK, PRAME, Survivin and TTK were selected as they showed the highest overlap across different cancer types and the most pronounced differential expression between tumor and matched normal tissue. While 31/52 (59.6%) patients showed an IFN-γ, only 11/52 (21.2%) patients exhibited an IL-2 response against at least one of the tested CTAs. Survivin was the CTA presenting the highest frequency of responses (18/52 IFN-γ and 5/52 IL-2 responses). PBMCs of patients with NSCLC showed the highest frequency of T-cell responses (83.3% with at least one IFN-γ response)

and patients with HCC and BCA the lowest proportion (40.0%).

Conclusions The observed findings underline the importance of TLS as a novel biomarker and a possible association to cellular responses may even enhance their prognostic value. Our planned analyses of combined humoral immune responses will further elucidate the role of TLS in anti-tumor immune response of the analyzed cancer types. A combined targeting of a predefined or personalized set of included TAAs appears promising across the different cancer types.

Disclosure Information E. Preugszat: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Koeln Fortune Program, Faculty of Medicine, University of Cologne. **M. Thelen:** None. **M. Garcia-Marquez:** None. **J. Lehmann:** None. **D. Keller:** None. **M. von Bergwelt-Baildon:** Other; Modest; Honoraria for advisory boards, for invited talks from BMS and financial support for research projects from Astellas, Roche and MSD. **H.A. Schlößer:** Other; Significant; Financial support for research projects from Astra Zeneca.

P06.03 BISPECIFIC ANTIBODY-DRIVEN SYNTHETIC AGONISTIC RECEPTOR ENGINEERED T CELLS LEAD TO SPECIFIC AND CONDITIONAL THERAPY IN MELANOMA CANCER MODELS

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10.1136/jitc-2021-ITOC.37

Background Immunotherapeutic approaches, including immune checkpoint blockade and adoptive T cell therapy (ACT) in the form of tumor-infiltrating lymphocytes (TIL), 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 said limitations, we propose a controlled ACT approach, where T cells are armed with synthetic agonistic receptors (SAR) 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 A375 and MV3 melanoma xenograft models. Further, we could show that SAR T cells exhibited resistance to MDSC-induced suppression of activation and proliferation.

Conclusions Here we apply the SAR x BiAb approach in efforts to deliver specific and conditional activation of SAR 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 MDSC-induced suppression, an interesting axis that requires further investigation.

Disclosure Information M. Benmebarek: None. **F. Märkl:** None. **J. Keyl:** None. **B. Loureiro Cadilha:** None. **M. Geiger:** None. **C. Karches:** None. **S. Endres:** None. **C. Klein:** None. **S. Kobold:** None. **A. Klüver:** None. **M. Schwerdtfeger:** None.

P06.04 TRANSCRIPTOME-WIDE NETWORK ANALYSIS PREDICTS THE ROLE OF LACTATE DEHYDROGENASE C IN BREAST CANCER CELL SURVIVAL AND IMMUNE DYSFUNCTION

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10.1136/jitc-2021-ITOC.38

Background Cancer testis antigens (CTAs) are lucrative anti-cancer targets given their restricted expression patterns and known roles as mediators of cancer hallmarks, including cancer metabolism, proliferation, survival, and cell motility. Lactate dehydrogenase C (LDHC) is a CTA with upregulated expression in poor prognosis subtypes of breast cancer, however its tumorigenic role is less understood. We recently reported that silencing LDHC reduces breast cancer cell survival through a dysregulated DNA damage response, thus highlighting its potential as an anti-cancer target with limited off-target effects. This study aimed to explore the changes in the transcriptome of breast cancer cells and immune-related mediators upon *in vitro* LDHC targeting.

Materials and Methods We silenced *LDHC* expression in breast cancer cell lines and investigated the downstream effects on the tumor cell transcriptome. Differentially expressed genes were subjected to regulatory network analyses. We further assessed the secretory profile of cytokines and immune checkpoint expression in *LDHC*-silenced cells and used the Tumor Immune Dysfunction and Exclusion (TIDE) algorithm to determine the effect of the interaction between *LDHC* expression and cytotoxic T lymphocyte (CTL) infiltration in the METABRIC breast cancer cohort.

Results Network analysis to investigate the effects of silencing *LDHC* on the tumor cell transcriptome identified 47 up- and 55 down-regulated transcripts (2.0-fold change, adj $p<0.05$). Differentially expressed genes in the *LDHC*-silenced cells were particularly enriched in canonical pathways regulating cell cycle checkpoint control, BRCA1-mediated DNA damage response and NF-kb signaling in response to infection. Upstream regulator analyses revealed the altered expression profile was associated with mTOR ($p=1.27e-5$, $z=2.242$) and CASP3 ($p=3.2e-4$, $z=2.250$) pathways, which in the presence of LDHC are predicted to activate TP53, Myc, NF-KB complex, STAT1/3, PRKC, CDK2, FOXO3 and HIF-1a while

inhibiting SMAD3, PTEN, ATM, IL18 and BCL2. Consequentially, the observed network-wide changes on LDHC silencing are predicted to negatively influence cellular growth and proliferation, cell migration and cell infiltration. The LDHC-associated network indicated a higher-level regulation by miR378a-3p (p=1.4e-7, z=-3.117), affecting the downstream mechanistic in LDHC-expressing cells. Interestingly, the miR378a causal network also indicated inhibition of the immune response in LDHC-positive cells. TIDE analysis indicated that high expression of LDHC in the METABRIC Her2 breast cancer cohort (TIDE score=1.97, p=0.049), and to a lesser extent in triple negative breast cancer (TIDE score=0.466, p=0.642), decreases the beneficial effect between CTLs and overall survival observed in LDHC Low tumors. Concurrently, *LDHC*-silenced cells displayed a pro-inflammatory gene expression and cytokine profile and down-regulated the expression of PD-L1 and Gal-9 immune checkpoints.

Conclusions Our findings provide an indication of potential CTL dysfunction in breast tumors with high LDHC expression and suggests that therapeutic targeting of LDHC may inhibit tumor growth while releasing the anti-tumor immune response in breast cancer.

Disclosure Information A. Naik: None. **J. Decock:** None.

P06.05 ENDOGENOUS T-CELL RESPONSES TO TEN MAJOR CANCER TESTIS ANTIGENS ARE FREQUENT IN ESOPHAGO-GASTRIC ADENOCARCINOMA AND ANTIGEN-SPECIFIC T CELLS CAN BE EXPANDED USING CD40-ACTIVATED B CELLS

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10.1136/jitc-2021-ITOC.39

Background Tumor-associated antigens (TAAs) and especially cancer testis antigens (CTAs) are classical tumor-specific targets for immunotherapies. As TAAs are shared between patients, strategies aiming to exploit these targets are scalable and potentially applicable across different types of cancer. Loss of target antigens and other mechanisms of immune escape have limited the success of CTA-directed immunotherapy. CAR T cells and other highly effective cellular therapies have renewed the interest in TAAs. Especially combined targeting of multiple antigens appears highly promising as recently shown in lymphoma. In our study, we aimed to characterize CTA-expression patterns and their impact on endogenous T-cell responses, T-cell abundance and antigen-presentation in esophago-gastric adenocarcinoma (EGA).

Materials and Methods 41 treatment-naïve EGA patients were included in our study. RNA of tumor and patient-matched healthy tissue was isolated and used for NanoString based RNA expression analysis of 26 CTAs and 25 genes associated with antigen-presentation. Based on CTA expression, 10 peptide pools were selected and co-cultured with peripheral blood mononuclear cells (PBMCs, n=21) to determine cellular anti-tumor immune responses in a FluoroSpot assay. T-cell abundance was assessed using immunohistochemistry (CD3, CD8) and digital image analyses of tumor area and invasive margin. Autologous CD40 activated B cells were used to expand antigen-specific T cells using peptide pools of CTAs.

Results NanoString analysis revealed pronounced differences regarding CTA expression, with CEP55 and MAGEA3/6 showing strong expression, while NY-ESO-1 or MAGEA1 were only weakly expressed. 68.3% (28/41) of the patients showed expression of $\geq$ 5/10 analyzed TAAs simultaneously. In line with the frequent expression, 75.0% of the patients showed a cellular response against at least one of the TAAs. T-cell responses were most frequently detected to Survivin and NY-ESO-1 (65.0% and 52.6% of patients, respectively), while only 20.0% responded to CEP55 or TTK peptide pools. Overall, 6/20 patients showed cellular responses against $\geq$5 TAAs simultaneously. We found a strong correlation of T-cell abundance and antigen-presentation. In addition, patients with a high Immune-Score showed increased TAA expression. Finally, we demonstrate feasibility of TAA-specific T-cell expansion using CD40 activated B cells as potential strategy to induce or enhance TAA immune responses in EGA.

Conclusions Our study highlights the importance of TAAs in EGA. The identified antigens are highly relevant for immunomonitoring of clinical trials and as targets for immunotherapy. Personalized immunotherapeutic strategies targeting EGA-specific or even patient specific TAAs appear highly promising in this challenging disease.

Disclosure Information M. Thelen: None. **M. Garcia-Marquez:** None. **J. Lehmann:** None. **D. Keller:** None. **E. Preugszat:** None. **M. von Bergwelt-Baildon:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Astellas, Roche, MSD. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; BMS. F. Consultant/Advisory Board; Modest; BMS. **H.A. Schlößer:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Astra Zeneca.

P06.06 ENHANCING TRAFFICKING AND RESISTANCE TO IMMUNOSUPPRESSION OF SYNTHETIC AGONISTIC RECEPTOR-TRANSDUCED T CELLS IN SOLID TUMOR MODELS

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10.1136/jitc-2021-ITOC.40

Background Chimeric antigen receptor therapy – although very efficacious in B cell malignancies – is facing many challenges which limit its success in solid tumors, e.g. on-target off-tumor toxicities, antigen heterogeneity, lack of T cell migration into tumors and an immunosuppressive tumor microenvironment. To better control on-target off-tumor effects and address antigen heterogeneity we developed a modular approach where we equipped T cells with a synthetic agonistic receptor (SAR). The SAR is only activated in the

presence of a bispecific antibody (BiAb) cross-linking the receptor with a tumor-associated antigen. While we could show efficacy of the SAR platform in different models, limited infiltration and immune suppression still hamper its function. We could previously demonstrate that T cell infiltration can be enhanced by transduction with carefully chosen chemokine receptors like CXCR6, CCR4 and CCR8. At the same time, gene silencing of checkpoint molecules like PD-1 can make T cells more resistant to immunosuppression, thus we assumed that combining these approaches might generate a desired T cell product.

Materials and Methods All constructs had been generated previously by overlap-extension cloning. The EGFRvIII (E3) SAR consists of extracellular EGFRvIII, transmembrane CD28 and intracellular CD28 and CD3ζ. Human CXCR6-GFP, CCR4-GFP and CCR8-GFP are composed of the chemokine receptors fused to GFP via a 2A sequence. Primary human T cells were retrovirally transduced to stably express the SAR and chemokine receptors. We analyzed migration, cytotoxicity and activation of the single and double (E3 SAR and chemokine receptor) transduced T cells. In addition, PD-1 was knocked out using CRISPR-Cas9 and killing kinetics of target cells and T cell activation were assessed.

Results Co-transduction with chemokine receptors significantly increased migration of E3 SAR T cells to their respective ligand while lysis of target-expressing tumor cell and T cell activation in the presence of BiAb were not affected *in vitro*. Additionally knocking out PD-1 enhanced killing kinetics and activation of E3 SAR and E3 SAR + CXCR6-GFP transduced T cells compared to corresponding mock electroporated T cells.

Conclusions Using the controllable and modular SAR – BiAb platform SAR T cell activation can be limited by stopping BiAb dosing if adverse events occur. In addition, SAR T cells can be redirected to an alternative tumor-associated antigen by exchanging the BiAb in the case of antigen escape. Here we present add-ons to this approach for increased tumor infiltration and resistance to immunosuppression. Since migration is enhanced upon co-transduction with chemokine receptors and target cell lysis is accelerated upon PD-1 knockout in vitro these two additional modifications seem very promising options to further improve tumor control in vivo.

Disclosure Information M. Schwerdtfeger: None. **M. Benmebarek:** None. **F. Märkl:** None. **C.H. Karches:** A. Employment (full or part-time); Significant; Daiichi Sankyo Deutschland GmbH. **A. Öner:** None. **M. Geiger:** A. Employment (full or part-time); Significant; Roche. **B. Cadilha:** None. **S. Endres:** None. **V. Desiderio:** None. **C. Klein:** A. Employment (full or part-time); Significant; Roche. **S. Kobold:** None.

P06.07 *IN VIVO* STUDIES OF IMMUNOMODULATORY A-CTLA-4 ANTIBODY IN A HUMANIZED MOUSE MODEL

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10.1136/jitc-2021-ITOC.41

Background Recent findings in cancer immunotherapy have reinforced the hypothesis that the immune system is able to control most cancers. Immunomodulatory antibodies can enhance immune responses, having the potential to generate anti-cancer immunity.[1–4]

Materials and Methods Most current studies addressing this question are performed in murine mouse model systems or use *in vitro* culture systems, which do not reflect the human in vivo situation, potentially leading to results that cannot be fully translated into human cancer therapy. Therefore, it is necessary to establish a new mouse model, which allows the study of cancer immunotherapy in the context of a human immune system. We focused on the establishment of a humanized mouse model, in which different immunomodulatory antibodies can be tested in the presence of a human immune system.

Results First experiments concerning the suitability to test immunomodulatory antibodies in the humanized mouse model, revealed that effects of checkpoint-control antibody a-CTLA-4 were similar to the effects seen in patients of clinical studies. To analyse the anti-tumor activities of immunomodulatory antibodies in vivo we are establishing a human melanoma-like tumor model in humanized mice.

Conclusions This enables us to test the efficacy of immunomodulatory agonistic antibodies (such as CP-870,893) and checkpoint control antibodies (such as anti-CTLA-4) in eliminating a melanoma-like tumor. Furthermore, parameters like tumor infiltrating human cells und cytokine/chemokine production can be analysed.

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Disclosure Information C. Reitinger: None. **F. Nimmerjahn:** None.

P07 Cell therapy in haematologic diseases

P07.01 A MODULAR AND CONTROLLABLE T CELL THERAPY PLATFORM FOR AML

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10.1136/jitc-2021-ITOC.42

Background Targeted immunotherapies have shown limited success in the context of acute myeloid leukemia (AML). The mutational landscape, 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 equipped with synthetic agonistic receptors (SARs) that are selectively activated only in the presence of a target AML-associated antigen, and a cross-linking tandem single chain variable fragment (taFv) 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 taFvs 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 antigens CD33 and CD123.

Results Anti-CD33-EGFRvIII and anti-CD123 EGFRvIII taFv, 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 taFv could form functional immunological synapses, resulting in efficient tumor cell lysis with specificity towards CD33-expressing AML cells. SAR-taFv combination could also mediate specific cytotoxicity against patient-derived AML blasts and leukemic stem cells whilst driving SAR T cell activation. In vivo, treatment with SAR-taFv combination could efficiently eradicate leukemia and enhance survival in an AML xenograft models. Furthermore, we could show selective activation of SAR T cells, as well as a controllable reversibility and modularity of said activation upon depletion of the T cell engaging molecule, both *in vitro* and *in vivo*.

Conclusions Here we apply the SAR-taFv platform in efforts to deliver specific and conditional activation of SAR-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 taFv facet. Moreover, we are able to circumvent pan-T cell activation due to the specific targeting and activation of SAR T cells.

Disclosure Information M. Benmebarek: None. **B. Loureiro Cadilha:** None. **M. Herrmann:** None. **S. Schmitt:** None. **S. Lesch:** None. **S. Stoiber:** None. **A. Darwich:** None. **C. Augsberger:** None. **B. Brauchle:** None. **M. Schwerdtfeger:** None. **A. Gottschlich:** None. **A. Gottschlich Rataj:** None. **N.C. Fenn:** None. **C. Klein:** None. **M. Subklewe:** None. **S. Endres:** None. **K. Hopfner:** None. **S. Kobold:** None.

P07.02 REGULATION OF CD19 CAR T- CELL ACTIVATION BASED ON ENGINEERED NUCLEAR FACTOR OF ACTIVATED T CELLS ARTIFICIAL TRANSCRIPTION FACTORS

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10.1136/jitc-2021-ITOC.43

Background CD19 CAR T- cells (Chimeric antigen receptor T cells that recognize CD19) present a therapeutic option for various malignant diseases based on their ability to specifically recognize the selected tumour surface markers, triggering immune cell activation and cytokine production that results in killing cancerous cell expressing specific surface markers recognized by the CAR. The main therapeutic effect of CAR is a specific T cell activation of adequate cell number with sequential destruction of tumorous cells in a safe therapeutic manner. In order to increase T cell activation, different activation domains were introduced into CAR. CAR T-cells are highly efficient in tumour cell destruction, but may cause serious side effects that can also result in patient death so their activity needs to be carefully controlled.[1] Several attempts were made to influence the CAR T cell proliferation and their activation by adding T cell growth factors, such as IL-2, into patients, however this approach of increasing the number of activating T cells with no external control over their number can again lead to non-optimal therapeutic effects. Different improvements were made by designing synthetic receptors or small molecule-inducible systems etc., which influence regulated expansion and survival of CAR T cells.[2]

Material and Methods In order to regulate CD19 CAR-T cell activity, different NFAT2 based artificial transcription factors were prepared. The full length NFAT2, one of the main players in T cell IL2 production, a key cytokine for T cell activation and proliferation was truncated by deletion of its own activation domain. Next, we joined via Gibson assembly tNFAT2$_{1\text{-}593}$ coding sequence with domains of different heterodimerization systems that interact upon adding the inductor of heterodimerization. The interaction counterparts were fused to a strong tripartite transcriptional activator domain VPR and/or strong repressor domain KRAB resulting in formation of an engineered NFAT artificial transcription (NFAT-TF) factors with external control. To determine the activity of NFAT-TF HEK293, Jurkat or human T cells were used.

Results Based on luciferase assay, carried out on NFAT-TF transfected HEK293 cells we first established that upon adding the external inductor of heterodimerization, efficient gene regulation occurs, according to VPR or KRAB domain appropriate functions. Findings were then transferred to Jurkat cells that were electroporated with appropriate DNA constructs, coding for NFAT-TF and CD19 CAR. After Raji:Jurkat co-culture ELISA measurements revealed that IL2 production and therefore CD19 CAR-T cell activity can be controlled by the action of NFAT-TF. The same regulation over the activity and subsequent proliferation status was also observed in retrovirally transduced human T-cells.

Conclusion We developed a regulatory system for therapeutic effect of CD19 CAR-T cells, a unique mechanism to control T cell activation and proliferation based on the engineered NFAT2 artificial transcription factor.

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Disclosure Information D. Lainšček: None. **V. Mikolič:** None. **Š. Malenšek:** None. **A. Verbič:** None. **R. Jerala:** None.

P07.03 'MYTCELL': A SMARTPHONE APPLICATIONGUIDES LOGISTICSAND MANAGEMENT OF CAR T-CELL & BITE RELATED TOXICITIES

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10.1136/jitc-2021-ITOC.44

Background Bispecific T-cell engagers (BiTE) and CD19-specific Chimeric Antigen Receptor (CAR) T-cell products are approved for relapsed and refractory B-cell neoplasms. However, rapid disease progression and the pre-treatment workflow during manufacturing challenges several specialities of health care professionals and involves a well educated team in the in-patient and out-patient setting. In addition, CARs and BiTEs are accompanied by a new spectrum of immune related toxicities. Currently, clinical trials investigate the safety of out-patient CAR T-cell administration, requiring high-level care during the early post-infusion period. To support the optimal management of these patients, we developed the interactive smartphone application 'myTcell', which guides and educates physicians in the pre-treatment logistics of CARs and BiTEs and management of related toxicities.

Materials and Methods We initiated a multi step content development process with an extensive literature research of toxicity guidelines consented by the ASTCT, SITC, NCCN and EBMT as well as of officially released drug information. Findings were translated into an information platform with diagnostic and therapeutic recommendations as well as algorithms for interactive toxicity grading tools. A prototype has been validated at five Geman treatment centers through a questionnaire, which measures the advantage over common guideline practice. 'myTcell' will become available as medicinal product class I for iOS, Android and desktop in Europe on 15th of July. App development has been funded through educational grants by Celgene, Gilead Sciences, Janssen and Novartis.

Results 'myTcell' guides disease and product specific in a step by step process through the clinical workflow of cell therapy. This includes recommendations for patient screening, safety assessment and stopping rules prior to leukapheresis and CAR T-cell transfusion. Upon entering relevant clinical data for grading of CRS, ICANS and HLH interactive tools display toxicity grade or likelihood of toxicity as well as grade-specific management. Further, 'myTcell' assists with the diagnosis and treatment of pancytopenia and infections. A map visualizes the availability of CAR T-cell therapy in Germany and links in-patient and out-patient care. Besides, 'myTcell' includes an overview of important publications and refers directly to respective PubMed abstracts.

Conclusions 'myTcell' has the potential to become a highly usable smartphone app supporting the application of T-cell recruiting immunotherapies as well as the assessment and treatment of novel immunotoxicities. In addition, it facilitates outreach and connects treatment centers and referrig physicians. Thus, 'myTcell' can translate into increased guideline adherence, accelerated broader and safer application of CARs and BiTEs and improved patient outcomes.

Disclosure Information V. Blumenberg: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Novartis, Gilead Sciences, Janssen, BMS/Celgene. **L. Siegmund:** None. **L. Fröhlich:** None. **M. Bergwelt:** None. **V. Bücklein:** None. **M. Subklewe:** None.

P08 Combination Therapy

P08.01 RATIONALE OF USING THE COMBINATION OF ANTI-PD-1 ANTIBODY AND ANTI-IL-8 ANTIBODY FOR THE PANCREATIC CANCER TREATMENT

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10.1136/jitc-2021-ITOC.45

Background Pancreatic ductal adenocarcinoma (PDAC) does not respond to immune checkpoint inhibitors (ICI) therapy as single agent treatments including anti-PD-1 antibody. One of the mechanisms for the resistance of PDAC to ICI is now attributed to the immunosuppressive microenvironment (TME) in PDAC. Myeloid cells are thought to be the predominant immunosuppressive cells in the TME. Human interleukin-8 (IL-8) is a pro-inflammatory chemokine in the CXC family and has the capability of recruiting myeloid cells into the TME to promote tumor progression and immune escape. Therefore, several anti-IL-8 blockade antibodies were developed including HuMax-IL8 and B108-IL8, which both are fully human IgG1 kappa monoclonal antibodies. We therefore tested whether anti-IL-8 antibodies can potentiate anti-tumor activity of anti-PD-1 antibody in a humanized model of PDAC.

Materials and Methods We reconstituted the immune system of the NGS mice with ex vivo activated human T cells and a combination of CD14+ and CD16+ myeloid cells after the mice were orthotopically implanted with human PDAC cells. 10x single nuclei RNA-Seq data processing was further performed to analyze differentially expressed genes among certain cell clusters.

Results Our results showed that anti-PD-1 antibody alone had a minimal anti-tumor activity when mice was reconstituted with ex vivo activated T cells. Interestingly, the infusion of the combination of CD14+ and CD16+ myeloid cells together with anti-PD-1 antibody resulted in a modest anti-tumor activity. Adding either HuMAX-IL8 or B108-IL8 led to a significantly enhanced anti-tumor activity. Both CD14+ and CD16+ myeloid cells appeared to be needed for the full anti-tumor activity of IL-8 blockade because mice infused with only CD14+ myeloid cells did not respond to IL-8 blockade and mice infused with only CD16+ myeloid cells responded partially to IL-8 blockade. This result suggested that the target of IL-8 is mainly present in CD16+ myeloid cells and is likely to be granulocytes. Tumor infiltrating immune cells were isolated and demonstrated that IL-8 blockade increases CD45 +CD11b+CD15+CD14- myeloid cells, which is known to comprise neutrophils and granulocytic myeloid derived suppressive cells (G-MDSC), in the tumors. Reconstitution of the mice with myeloid cells led to a decrease of CD8+ T cells in the tumors; however, IL-8 blockade brought the CD8+ T cell

number back to the baseline. Consistent with an effect of IL-8 blockade on the increase of CD15+CD14- myeloid cells, single nuclear RNA sequencing analysis of the tumor tissues showed that the innate immune response and cytokine response pathways in the myeloid cell cluster were activated by IL-8 blockade.

Conclusions This result suggested that IL-8 blockade did not simply inhibit myeloid cells as previously anticipated, but potentiated myeloid cells for the innate immune response and concomitant production of type I cytokines. Such immune responses may subsequently activate the effector T cells as the single nuclear RNA sequencing analysis demonstrated enhanced activation signals in the T cell cluster from the tumors treated by anti-IL-8 antibodies. Taken together, this study supports further testing of anti-IL-8 antibodies including B108-IL8 and HuMax-IL8 in combination with anti-PD-1 antibodies for PDAC treatment.

Disclosure Information P. Li: None. **N. Rozich:** None. **J. Wang:** None. **J. Gai:** None. **J. Wang:** None. **Y. Xu:** None. **B. Herbst:** None. **R. Yu:** A. Employment (full or part-time); Significant; NovaRock. **S. Muth:** None. **N. Niu:** None. **K. Li:** None. **V. Fune:** None. **A. Osipov:** None. **C. Wolfgang:** None. **M. Lei:** A. Employment (full or part-time); Significant; NovaRock. **T. Liang:** None. **L. Zheng:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Bristol-Meyer Squibb, Merck, iTeos, Amgen, NovaRock, Inxmed, Halozyme. E. Ownership Interest (stock, stock options, patent or other intellectual property); Significant; Alphamab, Mingruzhiyao. F. Consultant/Advisory Board; Significant; Biosion, Alphamab, NovaRock, Akrevia/Xilio, Ambrx, Novagenesis, Datarevive, Snow Lake Capitals, Mingruzhiyao. Other; Significant; Aduro Biotech.

P08.02 CCR2/CCR5 DUAL-ANTAGONIST 'LICENSES' THE RADIATION-INDUCED EFFECTOR T-CELL INFILTRATION IN THE ANTI-PD-1 ANTIBODY-TREATED PANCREATIC ADENOCARCINOMA

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10.1136/jitc-2021-ITOC.46

Background The resistance of pancreatic ductal adenocarcinoma(PDAC) to immune checkpoint inhibitors(ICIs) is mainly attributed to the immune-quiescent nature of its tumor microenvironment(TME). Radiotherapy(RT) activates innate responses including the RAGE and TLR2/4 pathways and subsequently modifies the TME by promoting the release of chemokines that recruit inflammatory cells into the TME. In this preclinical study, we examined the PDAC vaccine or RT as a T-cell priming mechanism together with BMS-687681, a small molecule dual-antagonist of CCR2 and CCR5(CCR2/5i) as an immunosuppressive TME-targeting agent, in combination with the anti-PD-1 antibody(αPD-1) as a new treatment.

Materials and Methods The hemi-spleen and Orthotopic mice model were used to investigate both GVAX and RT as T-cell priming agents in combination regimens that included αPD-1 and CCR2/5i. Dissected orthotopic pancreatic tumors were collected for analysis of tumor-infiltrating immune cells by flow cytometry. RNA from tumor-infiltrating immune cell pellets and whole-exome RNA sequencing was performed for further mechanism research.

Results CCR2 and CCR5 are associated with the immunosuppressive TME of PDAC patients and their expression were induced after treatment with GVAX+nivolumab. Using a mouse model of PDAC, we demonstrated that the addition of GVAX to CCR2/5i+αPD-1 combination therapy did not significantly improve antitumor activity. However, RT followed by αPD-1 and prolonged treatment with CCR2/5i conferred significantly better antitumor efficacy compared to the other combination treatments we studied. The combination of RT, αPD-1, and CCR2/5i enhanced intratumoral effector and memory T-cell infiltration. This combination suppressed Treg, M2-like TAM, and M-MDSC infiltration, but not M1-like TAM and PMN-MDSC infiltration. Finally, RNA sequencing showed that CCR2/5i partially inhibited RT-induced TLR2/4&RAGE signaling, which would have otherwise led to the release of immunosuppressive cytokines including CCL2 and CCL5. The inhibition of TLR2/4&RAGE signaling permitted the expression of effector T-cell chemokines such as CCL17 and CCL22.

Conclusions This study thus supports the clinical development of CCR2/5i in combination with RT and ICIs for PDAC treatment.

Disclosure Information J. Wang: None. **M. Tun Saung:** None. **K. Fujiwara:** None. **N. Niu:** None. **A. Narang:** None. **J. He:** None. **L. Zheng:** None.

P08.03 NEOANTIGEN CANCER VACCINE AUGUMENTS ANTI CTLA-4 EFFICACY

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10.1136/jitc-2021-ITOC.47

Background Immunotherapy based on anti CTLA-4 (αCTLA-4) and anti PD1 (αPD1) is being tested in combination with different therapeutic approaches including other immunotherapy approaches such as neoantigen cancer vaccines (NCV). Here we explored, in two cancer murine models, different therapeutic combinations of αCTLA-4 and/or αPD1 with a plasmid DNA vaccine expressing neoantigens and delivered by electroporation (EP).

Materials and Methods To evaluate the impact of NCV in the MC38 and in the CT26 tumor model three plasmid vaccines were generated with or without CD4 epitopes. Therapeutic DNA vaccines were delivered by EP in different therapeutic protocols including large tumors. Flow cytometry was utilized to measure CD8, CD4, T-reg, and B cells as well as neoantigen-specific immune responses, which were also measured by IFN-γ ELIspot.

Results Immune responses were augmented in combination with αCTLA4 but not with αPD1 in the MC38 tumor model with significantly impacting tumor growth. Similarly, neoantigen-specific T cell immune responses were observed in the CT26 tumor model where large tumors regressed in all mice treated with αCTLA-4 and NCV. In line with previous evidence, we observed an increased switched memory B cells in the spleen of mice treated with αCTLA-4 alone or in combination with NCV.

Conclusions These results support the use of NCV delivered by DNA-EP with αCTLA-4 and suggest a new combined therapy for clinical testing.

Disclosure Information F. Palombo: A. Employment (full or part-time); Significant; Neomatrix biotech. **E. Salvatori:** A. Employment (full or part-time); Significant; Takis biotech. **L. Lione:** A. Employment (full or part-time); Significant; Takis biotech. **M. Compagnone:** A. Employment (full or part-time); Significant; Neomatrix biotech. **A. Conforti:** A. Employment (full or part-time); Significant; Evvivax. **L. Aurisicchio:** A. Employment (full or part-time); Significant; Neomatrix biotech, Takis biotech, Evvivax.

P08.04 SUCCESSFUL IMMUNOTHERAPY OF THE BREAST CANCER METASTATIC DISEASE IN MICE USING A PHARMACEUTICAL TLR4-AGONIST INDUCES SYSTEMIC ANTI-TUMOR T CELL RESPONSE AND LONG-TERM T CELL MEMORY

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10.1136/jitc-2021-ITOC.48

Background A study of the anti-tumor T-cell response and immunological memory following successful 4T1 breast cancer immunotherapy with the combination of surgical resection of the primary tumor and subsequent macrophage/dendritic cell reprogramming using injections of the pharmaceutical TLR4-agonist.

Materials and Methods 15,000 cells of the 4T1 mouse breast carcinoma inoculated subcutaneously into BALB/c mice generated solid tumors and metastatic disease ended by the death of all the tumor-bearing animals during 30-40 days. Surgical resection of the primary tumor was performed on day 11. Pharmaceutical TLR4-agonist (Immunomax®) administered intraperitoneally in dose of 14 μg every 2-3 days, in total seven injections per course. Sorted macrophage/dendritic cells reprogramming was examined by RT-PCR. Tumor-reactive IFNγ-secretory T cells were counted using ELISPOT in ex vivo co-cultures of sorted CD4 T cells or CD8 T cells with the tumor lysate-loaded syngeneic dendritic cells or alive 4T1 tumor cells. Sorted CD8 effector T cell cytotoxicity was measured in their co-culture with different numbers of 4T1 target cells.

Results Using a combination of surgical resection of the primary 4T1 tumor and immunotherapy with the pharmaceutical TLR4-agonist for the treatment of metastatic disease in BALB/c mice a complete recovery of 20-30% mice was achieved. The complete responder mice effectively generated CD4 T cells and CD8 T cells, which specifically respond to 4T1 tumor antigens by IFN-production and kill 4T1 tumor cells in ex vivo co-cultures. The T-cell response is systemic, as tumor-specific T cells accumulate in the spleen. The second or third inoculation of the 4T1 tumor is accompanied by a complete absence of tumor growth in 50% and inhibition of tumor growth in the rest of the immune mice. An accumulation of significant numbers of T cells that respond to 4T1 tumor antigens by IFNγ-secretion, as well as of CD8 T cells that kill 4T1 tumor cells in a cytotoxic test was found in the secondary (tertiary) tumors, as well as in the draining lymph nodes. Immunological memory in complete responder mice that recovered due to the treatment with resection of the primary tumor and immunotherapy with a 4T1-agonist persisted for a long time (maximum observation period of 260 days).

Conclusions Macrophage/dendritic cell reprogramming with the TLR4-agonist for the post-resectional immunotherapy of 4T1 breast cancer metastatic disease induce tumor-specific CD4 and CD8 T cell responses and T-cell mediated long-living immune memory.

Funding This study was supported by the Russian Science Foundation (project no. 20-15-00391).

Disclosure Information E. Ushakova: None. **E. Lebedeva:** None. **A. Pichugin:** None. **R. Ataullakhanov:** None.

P08.05 COMBINED PHARMACOLOGICAL TARGETING OF ADENOSINE 2A- AND 2B-RECEPTOR ENHANCES CAR T CELL FUNCTION

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10.1136/jitc-2021-ITOC.49

Background Despite remarkable response rates mediated by anti-CD19 chimeric antigen receptor (CAR) T cells in selected B cell malignancies, CAR T cell therapy still lacks efficacy in the vast majority of tumors. A substantial limiting factor of CAR T cell function is the immunosuppressive tumor microenvironment. Among other mechanisms, the accumulation of adenosine within the tumor can contribute to disease progression by suppressing anti-tumor immune responses. Adenosine 2a- and 2b-receptor ($A2_A$ and $A2_B$)-mediated cAMP build-up suppresses T cell effector functions. In the present study we hypothesize, that combination therapy with the selective $A2_A$/$A2_B$ dual antagonist AB928 (etrumadenant) enhances CAR T cell efficacy.

Materials and Methods Second generation murine (anti-EPCAM) and human (anti-MSLN) CAR constructs, containing intracellular CD28 and CD3ζ domains, were fused via overlap extension PCR cloning. Murine or human T cells were retrovirally transduced to stably express the CAR constructs. $A2_A$/$A2_B$ signaling in CAR T cells was analyzed by phospho-specific flow cytometry of CREB (pS133)/ATF-1 (pS63). CAR T cell activation was quantified by flow cytometry and enzyme-linked immunosorbent assay (ELISA) of IFN-γ, IL-2 and TNF-α. CAR T cell proliferation was assessed by flow cytometry. CAR T cell cytotoxicity was assessed by impedance based real-time cell analysis.

Results AB928 protected murine CAR T cells from cAMP response element-binding protein (CREB) phosphorylation in the presence of stable adenosine analogue 5′-N-ethylcarboxamidoadenosine (NECA). NECA inhibited antigen-dependent CAR T cell cytokine secretion in response to four murine tumor cell lines. CAR T cell-mediated tumor cell lysis as well as proliferation were decreased in the presence of NECA or adenosine. Importantly, AB928 fully restored CAR T cell cytotoxicity, proliferation, and cytokine secretion in a dose dependent manner. Further, AB928 also restored antigen dependent cytokine secretion of human CAR T cells in the presence of NECA.

Conclusions Here we used the $A2_A/A2_B$ dual antagonist AB928 to overcome adenosine-mediated suppression of CAR T cells. We found that AB928 enhanced important CAR T cell effector functions in the presence of the adenosine analogue, suggesting that combination therapy with AB928 may improve CAR T cell efficacy. This study was limited to *in vitro* experiments. To confirm the relevance of our findings, this combination therapy must be further investigated in an *in vivo* setting.

Disclosure Information M. Seifert: None. **M. Benmebarek :** None. **B. Cadilha :** None. **J. Jobst:** None. **J. Dörr:** None. **T. Lorenzini:** None. **D. Dhoqina:** None. **J. Zhang:** None. **J. Zhang:** None. **U. Schindler:** E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Amgen Inc., Arcus Biosciences. Other; Significant; Arcus Biosciences. **S. Endres:** None. **S. Kobold:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Arcus Biosciences.

P08.06 COMBINING RIG-I-TARGETED IMMUNE ACTIVATION WITH CAR T CELL THERAPY INDUCES EFFICIENT TUMOR CONTROL IN MURINE PANCREATIC CANCER MODELS

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10.1136/jitc-2021-ITOC.50

Background The efficacy of chimeric antigen receptor (CAR) T cells against solid tumors remains unsatisfactory due to impaired trafficking of the CAR T cells into the tumor microenvironment (TME) and the presence of immunosuppressive factors and cells. 5'- triphosphate double-stranded RNA (3p-RNA) is recognized by the intracellular pattern recognition receptor retinoic acid-induced gene I (RIG-I). RIG-I activates a downstream signaling cascade, triggering the expression of type I interferons (IFN), proinflammatory cytokines and chemokines enhancing immune surveillance in the TME. We hypothesized that priming the TME with RIG-I ligands increases the efficacy of CAR T cell therapy.

Materials and Methods T110299 pancreatic tumor cells (derived from a genetically-engineered Kras and p53 mutant murine PDAC model) were engineered to express murine epithelial cell adhesion molecule (EpCAM) and used to induce subcutaneous or orthotopic tumors in C57BL/6J female mice. Mice bearing T110299 $EpCAM^+$ tumors were treated with intratumoral or i.v. injections of 3p-RNA followed by i.v. injection of syngeneic murine T cells that were retrovirally tranduced to express anti-EpCAM CARs. Three days after CAR T cell injection, immune cell composition and CAR T cell infiltration in the TME were assessed by flow cytometry. Additionally, tumor growth and survival were monitored.

Results Intratumoral injections of 3p-RNA reshaped the myeloid immune compartment in the TME by significantly reducing suppressive polymorphonuclear-MDSC and macrophages while increasing Ly6Chigh inflammatory monocytes. Moreover, antigen-presenting cells, such as dendritic cells and macrophages, were activated as evidenced by increased MHC-I expression levels. This was paralleled by a significant increase in the infiltration of CAR T cells into the TME in the combination therapy group. Interestingly, anti-EpCAM CAR T cells alone failed to control the tumor growth of T110299 $EpCAM^+$ tumors, while monotherapy with 3p-RNA slightly delayed tumor growth in the subcutaneous model. Combination of 3p-RNA with anti-EpCAM CAR T cells induced a significant clinical benefit with tumor regression in 50% of the treated mice in the subcutaneous tumor model and prolonged survival in an orthotopic model.

Conclusions Remodeling the immunosuppressive TME using RIG-I ligands is a promising strategy for overcoming therapeutic resistance of CAR T cells in solid tumors, such as pancreatic cancer.

Funding The project was supported by the international doctoral program 'i-Target: Immunotargeting of cancer' funded by the Elite Network of Bavaria and the Stiftungen zu Gunsten der Medizinischen Fakultät.

Disclosure Information A.M. Senz: None. **S.L. Formisano:** None. **B. Cadilha:** None. **T. Lorenzini:** None. **S. Endres:** None. **S. Kobold:** None. **M. Schnurr:** None. **L.M. König:** None.

P09 Young researcher session

P09.01 THE USE OF FDA APPROVED JAK, MTOR AND SRC INHIBITORS TO REGULATE T CELL-BISPECIFIC ANTIBODY-INDUCED CYTOKINE RELEASE WHILE NOT PREVENTING T CELL CYTOTOXICITY

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10.1136/jitc-2021-ITOC.51

Background T cell bispecific antibodies (TCBs) are potent T cell engagers, harboring a 2+1 format with one binder to the CD3ϵ chain and two binders to specific tumor antigens. Crosslinking of CD3 with tumor antigens triggers T cell activation and proliferation, cytokine release and tumor cell killing. TCB treatment is sometimes associated with safety liabilities due to on-target on-tumor or on-target off-tumor cytotoxicity and cytokine release. Off-tumor activity of the TCB may occur if the targeted tumor antigens are expressed on healthy cells, which may potentially result in tissue damages and compromise the patient's safety. Patients treated with TCBs may also experience a Cytokine Release Syndrome (CRS), characterized by fever, hypotension and respiratory deficiency and associated with the release of pro-inflammatory cytokines such as IL-6, TNF-α, IFN-γ, and IL-1β. Tyrosine kinases such as Src, mTOR and JAK1/2 are involved in downstream signaling pathways after engagement of the T cell receptor.

Materials and Methods 52 FDA approved kinase inhibitors were screened in the presence of T cells activated on CD3 coated plates, mimicking TCB stimulation. Src, mTOR and JAK inhibitors were selected based on their capacity to prevent both, cytokine release and T cell proliferation. Using an *in vitro* model of target cell killing by human peripheral blood mononuclear cells stimulated with TCBs, we validated the effects of mTOR, JAK and Src kinase inhibitors on TCB-induced T cell activation, tumor cell killing and cytokine release. *In vivo*, the effect of mTOR, JAK and Src kinase inhibitors on TCB-induced cytokine release was confirmed in humanized NOD scid gamma (NSG) mice engrafted with human hematopoietic stem cells and treated with CD19-TCB.

Results In line with previous reports for CAR-T cells, dasatinib (a src inhibitor) was found to fully switch off TCB-induced T cell functionality as well as the other src inhibitors bosutinib and ponatinib. In contrast, temsirolimus, sirolimus and everolimus (mTOR inhibitors) and ruxolitinib, baricitinib, tofacitinib, and fedratinib (JAK1/2 inhibitors) were found to more potently prevent TCB-induced cytokine release without blocking TCB-mediated target cell killing.

Conclusions These results provide evidence that the mechanisms of TCB-dependent cytokine release and tumor cell killing can be uncoupled. The FDA-approved mTOR and JAK1/2 inhibitors could potentially be used to mitigate CRS whereas the Src inhibitor dasatinib could rather stand as a potential antidote for on-target off-tumor activity or high-grade CRS.

Disclosure Information G. Leclercq: A. Employment (full or part-time); Modest; Roche. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Roche. **H. Haegel:** A. Employment (full or part-time); Modest; Roche. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Roche. **A. Schneider:** A. Employment (full or part-time); Modest; Roche. **A. Giusti:** A. Employment (full or part-time); Modest; Roche. **V. Pulko:** A. Employment (full or part-time); Modest; Roche. **A. Toso:** A. Employment (full or part-time); Modest; Roche. **T. Zimmermann:** A. Employment (full or part-time); Modest; Roche. **L. Green:** A. Employment (full or part-time); Modest; Roche. **N. Steinhoff:** A. Employment (full or part-time); Modest; Roche. **J. Sam:** A. Employment (full or part-time); Modest; Roche. **M. Bacac:** A. Employment (full or part-time); Modest; Roche. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Roche. **P. Umaña:** A. Employment (full or part-time); Modest; Roche. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Roche. **C. Klein:** A. Employment (full or part-time); Modest; Roche. E. Ownership Interest (stock, stock options, patent or other intellectual property); Modest; Roche.

P09.02 EPIGENETIC MODULATION OF NEUROBLASTOMA ENHANCES T- AND NK CELL IMMUNOGENICITY VIA INDUCTION OF SURFACE EXPRESSION OF MHC CLASS I AND MICA/MICB

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10.1136/jitc-2021-ITOC.52

Background Neuroblastoma (NBL) is the most common pediatric solid tumor and responsible for about 15% of all pediatric cancer deaths. The majority of high-risk (HR) patients suffers from relapse after intense therapy regimens, resulting in a 5-year survival rate of only 40%. Even though the potential of immune interference in HR-NBL is shown by the additive effect of anti-GD2 monoclonal antibody therapy to the treatment protocol, long-term follow-up studies reveal that the beneficial effect of immunotherapy diminishes over time. We hypothesize that this is a result of inadequate (adaptive) immune engagement caused by the extensive immunomodulatory capacity of HR-NBL and its microenvironment. One of the most remarkable immunomodulatory strategies of NBL tumors is the absence of MHC-I surface expression, thereby preventing cytotoxic T cell recognition and killing. MHC-I lacking cells are known to be subjected to NK cell mediated cytotoxicity, however, we have shown that NBL is able to evade this by temporary upregulating surface expression of MHC-I, thereby becoming temporarily more prone to T cell mediated cytotoxicity. The aim of this project is to identify pharmacological strategies to enhance adaptive immune activation and therewith immunogenicity of HR-NBL.

Materials and Methods FDA-approved drug libraries were screened to identify compounds enhancing MHC-I surface expression in NBL cell lines using high-throughput flow cytometry analyses optimized for adherent NBL cells. The effect of positive hits was subsequently confirmed in a panel of NBL patient-derived tumeroids. Alterations in the transcriptome and translatome upon incubation with compounds of interest were further studied to identify potential additional immunomodulatory effects in NBL. Ultimately, compound treated NBL cell lines and tumeroids were co-cultured with PRAME reactive tumor-specific T cells and healthy-donor NK cells to determine the *in vitro* effect on T- and NK cell cytotoxicity.

Results Drug library screening revealed MHC-I upregulation upon treatment of NBL cell lines and patient-derived tumeroids with multiple histon deacetylase inhibitors (HDACi). Further investigation of immunomodulatory effects of HDACi in NBL revealed enhanced expression of several additional players of the antigen presenting machinery, immunoproteasome expression, and MICA/MICB upregulation in NBL cells. We show that in untreated NBL cells, plasticity of MHC-I expression causes evasion of both NK- and T cell mediated cytotoxicity. Intriguingly, co-culture of NBL cells with tumor-specific T cells and healthy-donor NK cells upon treatment with the HDACi Entinostat resulted in enhanced *in vitro* T- and NK cell activation and cytotoxicity.

Conclusions We show pharmacological upregulation of MHC-I, other antigen presenting machinery players, and the NKG2D ligands MICA/MICB upon HDACi in HR-NBL. Pretreatment of NBL with HDACi resulted in enhanced *in vitro* T- and NK cell mediated cytotoxicity, substantiating HDACi as a potential strategy to improve adaptive immune engagement and therewith immunogenicity to aid NBL treatment.

This work was supported by the Villa Joep Foundation [IWOV-Actief.51391.180034].

Disclosure Information A.M. Cornel: None. **D.A.M.H. van den Beemt:** None. **E. Dunnebach:** None. **M.P. van Dierselhuis:** None. **S. Nierkens:** None.

P09.03 HYALURONIC ACID AS A NEW IMMUNOLOGIC ADJUVANT IN CANCER: DESIGN OF EFFECTIVE PREVENTIVE AND THERAPEUTIC VACCINATION STRATEGIES FOR HER2/NEU-POSITIVE BREAST TUMORS

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10.1136/jitc-2021-ITOC.53

Background The use of proteins as immunogens is attractive for the development of vaccines, but requires efficient adjuvants to overcome their weak immunogenicity. Recently, we investigated the potential of the TLR2/4 agonist hyaluronan (HA) as an immunological adjuvant for protein-based vaccines.[1] [2] Conjugation of HA to antigens strongly increased

their immunogenicity and promoted their rapid translocation to draining lymph nodes, resulting in robust and long-lasting humoral responses.[1] On these bases, we investigated the potentiality of HA-based technology in the design of cancer vaccines. To this aim, HA was conjugated to the extracellular domain of rat HER2/neu (rHER2/neu) and validated in the preventive and therapeutic vaccination settings.

Materials and Methods Female BALB/c or BALB-neuT mice were immunized with rHER2/neu-HA. In vivo depletion of $CD4^+$, $CD8^+$ T and B cells was performed, and sera and spleens were collected to characterized antigen-specific humoral and cellular responses. Vaccinated BALB/c mice were challenged and re-challenged with rHER2/neu-overexpressing TUBO cells to assess the protective or therapeutic activity of rHER2/neu-HA vaccination strategy, as well as immunological memory.

Results HA performed efficiently as robust and long-lasting humoral (IgG1, IgG2a, and IgG2b) and cellular responses were detected using very low antigen doses and number of boosters. Outstandingly, at 1-year post-vaccination, anti-rHER2/neu specific antibodies showed even improved effector functions (maturation of affinity for the receptor and increased complement-derived cytotoxicity functions). HA vaccination turned out effective in both the prophylactic (100% mice survived) and therapeutic (tumor regression in 2/12 mice) settings, and broke tolerance against rHER2/neu, delaying spontaneous tumor growth in BALB-neuT mice. Both humoral and cellular responses contributed to the success of HA-based vaccination, but $CD8^+$ T cells played only a marginal role.

Conclusions Cancer vaccines have not yet achieved significant clinical efficacy due to their poor immunogenicity, and the validation of more effective adjuvants occurred sometimes at the expense of safety. HA combines the unique immunomodulatory features of a TLR agonist with the tolerability of a fully natural polymer, proving to be a promising adjuvant for the creation of effective and safe cancer vaccines with the potential for rapid clinical translation.

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Disclosure Information D. Carpanese: None. **I. Montagner:** None. **A. Dalla Pietà:** None. **V. Rossi:** None. **A. Penna:** None. **G. Zuccolotto:** None. **G. Pasut:** None. **A. Grigoletto:** None. **A. Rosato:** None.

P09.04 IMPACT OF MAJOR ONCOLOGIC SURGERY ON IMMUNE RESPONSES IN THE IMMEDIATE POST-OPERATIVE SETTING IN OESOPHAGEAL ADENOCARCINOMA PATIENTS; A GUIDE TO HARNESSING THE DOUBLE-EDGED SWORD OF CANCER SURGERY

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10.1136/jitc-2021-ITOC.54

Background Immune checkpoint inhibitors (ICIs) are being investigated for their role as an adjunct in the multimodal treatment of oesophageal adenocarcinoma (OAC). The most appropriate time to incorporate ICIs remains unknown. Our study profiles systemic anti-tumour immunity perioperatively to help inform the optimal timing of ICIs into current standards of care for OAC patients.

Methods Systemic immunity in 11 OAC patients was phenotyped prior to oesophagectomy and on post-operative days (POD) 0, 1, 3, 7 and week 6 using flow cytometry. Longitudinal serological profiling was conducted by 54-plex-ELISA. The frequency of circulating lymphocytes, T cells, T helper cells and cytotoxic T lymphocytes was profiled longitudinally. The activation status of T cells was also assessed using CD69, CD27, CD62L and CD45RA as well as the proportion of T cell subsets in circulation, which included: naïve, central memory, effector memory and terminally differentiated effector memory T cells. This study also profiled the longitudinal alteration of immune checkpoint expression on circulating T cells, which included: PD-1, CTLA-4, TIGIT, TIM-3, LAG-3, PD-L1 and PD-L2. Damage-associated molecular patterns (calreticulin, HMGB1 and MIC-A/B) were also assessed.

Results The frequency of naïve T cells increased in circulation post-oesophagectomy from POD-0 to POD-7 ($p<0.01$) but returned to baseline at week 6. Effector memory T cells had decreased by POD7 but increased substantially by week 6 ($p<0.05$). A steady increase in activated circulating CD27+ T cells was observed from POD-0 to POD-7 ($p<0.05$). The percentage of PD-1+ and CTLA-4+ T cells peaked on POD-1 and was substantially decreased by week 6 ($p<0.01$). Th1 cytokines were decreased in the immediate post-operative setting with a reduction in IFN-Y, IL-12p40, CD28, CD40L and TNF Alpha. In addition to this IP-10 aka cxcl-10 which is an important chemokine ligand in recruiting anti-tumour TH1 cells and polarising the immune response to a Th1 phenotype is significantly reduced perioperatively. There is a simultaneous increase in Th2 cytokines in the immediate post-operative setting with a significant increase in IL4, IL10, IL16, IL1RA and MCP1 before returning to preoperative levels at week 6.

Conclusion Our study highlights the prevailing immunophenotype and responses to surgery with a switch in balance towards a Th2 and potentially M2 phenotype and consequently, an immunosuppressive milieu. Therefore, orchestrating M2 reprogramming toward an M1 phenotype and similarly shifting the balance in favour of a Th1 phenotype would offer a potent therapeutic approach for augmenting tumourigenesis and promoting cancer regression. Consequently, this study paves the way for further studies and appropriate trial design are needed to interrogate the use of ICB as a trimodal approach with chemoradiotherapy and chemotherapy alone for locally advanced disease in the neoadjuvant and adjuvant setting to determine the optimal timing and subset of patients for their use in the era of precision targeted therapies.

Disclosure Information N.E. Donlon: None. **M. Davern:** None. **A. Sheppard:** None. **F. O'Connell:** None. **S. Ramjit:** None. **C. Hayes:** None. **M. Mc Clean:** None. **H. Temperley:** None. **C. Butler:** None. **N. Ravi:** None. **C. Donohoe:** None. **J. O' Sullivan:** None. **M.R. Dunne:** None. **J.V. Reynolds:** None. **J. Lysaght:** None.

P09.05 PLASMA CD27, A SURROGATE OF INTRATUMORAL CD27-CD70 INTERACTION, CORRELATES WITH IMMUNOTHERAPY RESISTANCE IN RENAL CANCER

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10.1136/jitc-2021-ITOC.55

Background CD70, a costimulatory molecule on antigen presenting cells, is known to activate CD27-expressing T cells. CD27-CD70 interaction leads to the release of soluble CD27 (sCD27). However, persistent interaction of CD27 and CD70 such as in chronic infection may exhaust the T cell pool and promote apoptosis. Surprisingly, our analysis based on TCGA database show that clear cell renal cell carcinoma (ccRCC) expresses the highest levels of CD70 among all solid tumors. Despite the important clinical efficacy of immunotherapy by anti-PD-1 in RCC patients, the overall response to anti-PD1 remains modest. The relationship between the CD27-CD70 interaction in the RCC and the response to immunotherapy is still unclear.

Materials and Methods To study the CD27 and CD70 expression in the tumor microenvironment (TME), FFPE tumor tissues from 25 RCC patients were analysed using multiplex *in situ* immunofluorescence. 10 fresh RCC tumor samples were collected to analyse the phenotype of $CD27^+$ T cells by flow cytometry and 4 samples were proceeded for single-cell RNA-seq analysis. A cohort of metastatic RCC patients (n = 35) treated by anti-PD-1 were enrolled for the measurement of plasma sCD27 by ELISA and the survival analysis is also realized.

Results In the TME, we demonstrated that $CD27^+$ T cells interact with CD70-expressing tumor cells. In fresh tumors from RCC patients, $CD27^+$ T cells express higher levels of cleaved caspase 3 (a classical marker of apoptosis) than $CD27^-$ T cells. We confirmed the apoptotic signature (BAX, FASLG, BCL2L11, CYCS, FBXO32, LGALS1, PIK3R1, TERF1, TXNIP, CDKN2A) of $CD27^+$ T cells by single-cell RNAseq analysis. $CD27^+$T cells also had a tissue resident memory T cell phenotype with enriched gene expression of ITGAE, PRDM1, RBPJ and ZNF683. Moreover, $CD27^+$T cells display an exhaustion phenotype with the expression of multiple inhibitory receptors gene signature (PDCD1, CTLA4, HAVCR2, LAG3, etc). Besides, intratumoral CD27-CD70 interaction significantly correlates with plasma sCD27 concentration in RCC ($p = 0.0017$). In metastatic RCC patients treated with anti-PD-1, higher levels of sCD27 predict poor overall survival ($p = 0.037$), while it did not correlate with inflammatory markers or clinical prognostic criteria.

Conclusions In conclusion, we demonstrated that sCD27, a surrogate of T cell dysfunction in tumors likely induced by persistent interactions of $CD27^+$T cells and CD70-expressing tumor cells, is a predictive biomarker of resistance to immunotherapy in mRCC. To our knowledge, this is the first report showing that a peripheral blood biomarker may reflect certain aspects of the tumor-host interaction in the tumor microenvironment. Given the frequent expression of CD70 and CD27 in solid tumors, our findings may be further extended to other types of tumors. CD70-CD27 interaction could thus be considered as a mechanism of tumor escape, but also a novel therapeutic target in cancers.

Disclosure Information N. Benhamouda: None. **I. Sam:** None. **N. Epaillard:** None. **A. Gey:** None. **A. Saldmann:** None. **J. Pineau:** None. **M. Hasan:** None. **V. Verkarre:** None. **V. Libri:** None. **S. Mella:** None. **C. Granier:** None. **C. Broudin:** None. **P. Ravel:** None. **B. Jabla:** None. **N. Chaput:** None. **L. Albiges:** None. **Y. Vano:** None. **O. Adotevi:** None. **S. Oudard:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; SIRIC CARPEM, FONCER. **E. Tartour:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Fondation ARC, INCA PLBio, Labex Immuno-Oncology, SIRIC CARPEM, FONCER, IDEX université de Paris, Inserm Transfert.

P09.06 INVESTIGATING VARIOUS PATIENT PARAMETERS AS PROGNOSTIC MARKERS FOR PATIENTS WITH ADVANCE STAGE NASOPHARYNGEAL CARCINOMA UNDERGOING INDUCTION CHEMOTHERAPY FOLLOWED BY EPSTEIN-BARR VIRUS CYTOTOXIC T-LYMPHOCYTE IMMUNOTHERAPY

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10.1136/jitc-2021-ITOC.56

Background Previous prospective phase II study conducted by our research group at the National Cancer Centre Singapore had shown the efficacy of combined induction chemotherapy followed by cytotoxic T-lymphocyte (CTL) immunotherapy as a first-line treatment for advance nasopharyngeal carcinoma (NPC) – i.e. median survival for patients treated with combined therapy was 29.9 months, compared to 17.7 months for patients who received only standard chemotherapy.[1] Using the same data set, we further investigate the correlation between various patient factors (Eastern Cooperative Oncology Group (ECOG) score, gender, age, initial stage of cancer, neutrophil-to-lymphocyte ratio (NLR), initial EBV-DNA titre) on overall survival (OS). This is to further validate our hypothesis that the improved OS is due to an effect of treatment and not due to intrinsic patient factors.

Materials and Methods Survival distribution curves were estimated using the Kaplan-Meier method and differences were compared statistically using log-rank test. IBM SPSS statistics software package (v. 22) was used for the purpose of statistical analysis. Overall survival was defined as time from diagnosis to date of event (date of death/date of last follow-up). For analysis of overall survival, data for patients who were alive or who were lost to follow-up were censored at the end of study period.

Results It was revealed that lower ECOG score, a scale used to assess the physical condition of patients, correlated with longer OS while other characteristics such as gender, age, initial stage of cancer, NLR, and initial EBV-DNA titre did not

correlate with survival outcomes. ECOG0 patients had a median survival of 146.7 weeks, compared to ECOG1 patients, which had a median survival of 86.6 weeks (hazard ratio: 0.35; 95% CI: 0.14-0.84; P = 0.033).

Conclusions Even though ECOG performance status is found to be statistically associated with survival outcome of patients with advance stage NPC. This result is unsurprising as the prognostic value of ECOG has been well documented in literature, albeit in other cancer types. Other patient parameters such as gender, age, initial stage of cancer, NLR, and initial EBV titre, did not yield significance and did not prognosticate for survival outcome. This finding supports our hypothesis that the improved survival outcomes observed in advance NPC patients treated with chemotherapy followed by EBV CTL-immunotherapy is due to effects of treatment and not because of intrinsic patient factors.

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Disclosure Information A. Chu: None. **S. Han:** None. **H. Toh:** None.

P09.07 NOVEL INSIGHTS INTO IMMUNE-INDEPENDENT FUNCTIONS OF IMMUNE CHECKPOINT INHIBITORS IN OESOPHAGEAL ADENOCARCINOMA; POTENTIAL IMPLICATIONS FOR DESIGNING COMBINATION IMMUNO-CHEMOTHERAPY REGIMENS TO ACHIEVE SYNERGISTIC RESPONSES

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10.1136/jitc-2021-ITOC.57

Background Immune checkpoint inhibitors (ICIs) reinvigorate anti-tumour immunity in oesophageal adenocarcinoma (OAC). However, emerging studies have identified novel immune-independent functions for immune checkpoints (ICs) in other solid tumour-types, whereby IC-intrinsic signalling in gastric cancer cells confers chemoresistance. This study explores immune-independent functions of ICs in OAC and if therapeutic blockade may enhance chemotherapy toxicity.

Materials and Methods OAC cells were screened *in vitro* and *in vivo (n=14 OAC human tissue biopsies)* for a range of ICs (PD-1,TIGIT,TIM-3,LAG-3,A2aR,PD-L1,PD-L2,CD160) by flow cytometry. The phenotype of OAC cells expressing ICs was also assessed for features of stemness (ALDH, CD54), senescence (β-galactosidase) and invasiveness (vimentin) in the absence and presence of chemotherapy by flow cytometry. OAC cells were also treated with chemotherapy in the absence and presence of a MEK inhibitor to determine if MEK signalling regulated IC expression. Importantly, the effect of ICIs on the hallmarks of cancer in OAC cells was assessed which included: OAC cell viability (CCK-8 assay and western blot to assess Bcl-xL and Bcl-2 levels), proliferation (BrdU assay and ki67 expression by intracellular flow cytometry), chemo-sensitivity (annexin-V propidium iodide assay and cell cycle analysis by flow cytometry and expression of chemotherapy efflux and influx pumps by western blot: ATP7a, ATP7b, CTR1 and ABCB9), metabolism (seahorse), invasiveness and stemness characteristics (vimentin and aldefluor assay, respectively by flow cytometry) and DNA repair (γH2ax by flow cytometry to assess levels of DNA repair and the expression of DNA repair genes were quantified by qPCR: MLH1, SMUG1, PARP1, MMS19) was assessed in OAC cells.

Results A subpopulation of stem-like, senescent and vimentin$^+$ OAC cells were enriched for ICs, which was enhanced by FLOT and CROSS chemotherapy regimens. IC expression increased on the surface of OAC cells 48h post-chemotherapy treatment and was sustained up to 3 weeks post-treatment *in vitro*. Inhibition of pro-survivla MEK signalling reduced chemotherapy-induced upregulation of ICs. Blockade of PD-1, TIGIT, A2aR, TIM-3 and PD-L1 decreased proliferation, DNA repair, induced apoptosis and enhanced toxicity of FLOT in OAC cells. Blockade of TIGIT decreased pro-survival Bcl-xL factor, induced cell death and promoted a more glycolytic phenotype in OAC cells.

Conclusions Several novel ICs have been identified as potential targets to enhance chemotherpay efficacy in OAC. Upregulation of ICs on OAC cells following chemotherapy may represent potential mechanisms of chemo-immune resistance for stem-like, senescent and vimentin$^+$ aggressive cancer cell clones. Combining ICIs with chemotherapy may synergise with chemotherpay in OAC patients via immune-independent mechansims and boost response rates to current standards of care. Further studies are warranted through clincal trials to further establish synergistic ICI-chemotherapy combinations in OAC.

Disclosure Information M. davern: None. **C. Buckley:** None. **C. Fitzgerald:** None. **A.B. Heeran:** None. **N.E. Donlon:** None. **J. McGrath:** None. **R. O' Brien:** None. **F. O' Connell:** None. **B. Murphy:** None. **N. Lynam-Lennon:** None. **J.V. Reynolds:** None. **S.G. Maher:** None. **A.D. Sheppard:** None. **A. Bhardwaj:** None. **A. Bhardwaj:** None. **C. Butler:** None. **N. Ravi:** None. **J. Lysaght:** None.

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