Conclusions Collectively, our findings indicate that locally injected CAR T-cells may safely induce relevant anti-tumor effects in brain metastases from lung cancer. Additional anti-angiogenic treatment provides a mechanism to enhance intratumoral CAR T-cell persistence and reduce tumor growth.

C. Eberle: None. **J. Blobner:** None. **L. Dengler:** None. **J.J. Herold:** None. **A. Mühlbauer:** None. **T. Xu:** None. **K.J. Müller:** None. **N. Teske:** None. **P. Karschnia:** None. **S. Langer:** None. **H. Ishikawa-Ankerhold:** None. **M. Subklewe:** None. **N. Thon:** None. **J. Tonn:** None. **V.R. Buchholz:** None. **L. von Baumgarten:** None.

10.03 LONG LASTING ALTERATIONS OF THE IMMUNOPHENOTYPE AND CYTOKINE SIGNATURE OF DLBCL SURVIVORS RESULTS IN PERSISTING IMMUNE DYSFUNCTION

G Benintende*, R Pelzl, K Wendland, F Gsottberger, S Petkovic, L Mellenthin, S Völkl, A Mackensen, F Müller. *Universitätsklinukum Erlangen, Erlangen, Germany*

10.1136/jitc-2024-ITOC10.8

Background Immune cell alterations in active neoplasms are established by cancer cells and result from a complex crosstalk between cancer, immune, vascular, and stromal cells, referred to as tumor microenvironment (TME). Myeloid-derived suppressor cells (MDSC) are a myeloid subpopulation, integral to immunosuppression in the TME. In diffuse large B cell lymphoma (DLBCL), high MDSCs correlate with stage and are associated with poor outcome. Despite increasing understanding of the TME in active disease, the status of the immune system in complete remission (CR) and practical consequences thereof have not been studied.

Materials and Methods We established a flow cytometry-based immunophenotype analysis of fresh blood samples to compare patients at first diagnosis of DLBCL, cured from DLBCL, and healthy donors. Inhibitory MDSCs were shown by T cell suppression assays and intracellular staining of Arg1 and COX2. Functional characterization was done by T cell vaccine recall response following in vitro stimulation with SARS-CoV2 peptide. Serum cytokines were measured by ELISA and correlated with immunophenotypic data.

Results Patients cured from DLBCL have persistent immune dysfunction. Specifically, rate and number of MDSCs are significantly elevated in cured patients even years after remission has been achieved. These findings are not affected by age, stage, R-IPI, therapy lines, or time elapsed since end of therapy. The suppressive capacity of MDSC is confirmed by inhibition of T cells proliferation in co-culture assay and by high intracellular expression of Arg1 and COX2. The persistence of inhibitory myeloid cells in the cured patients is accompanied by additional changes in immune cells and cytokines. The CD4 and CD8 T cell compartments are substantially activated, with a consistent increase of activated CD4 T cells (HLA-DR and CD69+) and of terminally differentiated T cells such as CD8 TEMRA (CD45RA+/CD27-). The antigen specific response to SARS-CoV2 peptide in the cured cohort is lower than that of the age-matched controls. Further supporting chronic inflammation, several cytokines including IL6 or β2-microglobulin remain elevated in the cured, while B NHL-derived CXCL9 or CXCL10 are back to levels of healthy controls. In line with chronic inflammation, the number of MDSCs positively correlates with levels of serum IL-6. This is further linked through the demonstration that monocytes of healthy individuals shift towards MDSC phenotype when stimulated with IL-6 *in vitro*.

Conclusions Altogether, the presence of inhibitory myeloid cells and hyperactivated senescent T cells, supports a persisting systemic immune dysfunction in DLBCL survivors, which show functional relevance and can impact the patients' management. Whether these changes predict relapse or long-term responses is under ongoing investigation.

G. Benintende: B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Kite/Gilead. **R. Pelzl:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Kite/Gilead. **K. Wendland:** None. **F. Gsottberger:** None. **S. Petkovic:** None. **L. Mellenthin:** None. **S. Völkl:** None. **A. Mackensen:** None. **F. Müller:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Modest; Kite/Gilead.

10.04 SPATIAL TRANSCRIPTOMICS IDENTIFIES METABOLIC DYSREGULATION AS A KEY DRIVER OF T CELL EXCLUSION IN ESOPHAGEAL ADENOCARCINOMA

[1,2]J Sanders*, [1,2]EN Bos, [3]N Hahn, [4]I Nijman, [1]HWM van Laarhoven, [1]DL van der Peet, [3]J Van den Bossche, [1]TD de Gruijl, [1,2]S Derks. *[1]Amsterdam UMC location Free University, Medical Oncology; Cancer Center Amsterdam, Cancer Treatment and Quality of Life, Amsterdam, Netherlands; [2]Oncode Institute, Utrecht, Netherlands; [3]Department of Molecular Cell Biology and Immunology, Amsterdam Cardiovascular Sciences, Amsterdam Gastroenterology Endocrinology Metabolism, Amsterdam Institute for Infection and Immunity, Amsterdam UMC location Free University, Amsterdam, Netherlands; [4]Utrecht Sequencing Facility, Utrecht, Netherlands*

10.1136/jitc-2024-ITOC10.9

Background Success of neoadjuvant chemoradiotherapy (nCRT) in esophageal adenocarcinoma (EAC) is dependent on the level of activation of the tumor immune microenvironment (TIME). Patients with a complete pathological response ((pCR), 20% of EACs) have higher intratumoral CD8 T cell levels and a higher CD8:CD163 ratio compared to patient with a non-pCR. To improve response to nCRT we have to identify and target the mechanism EACs use to keep CD8 T cells out.

Materials and Methods To this end we used tissues of patients with high vs. low intratumoral CD8 T cell density (multiplex immunohistochemistry) from a previous study for spatial whole transcriptomics (Nanostring GeoMx DSP) to characterize the transcriptome of cancer cells in CD8 high vs. low tumor areas. Regions of interest (ROI) were determined based on T cell density (CD3+) and analyzed for whole transcriptome of both tumor cells (PanCK+) and adjacent immune cells (CD45+) separately. Transcriptional differences were validated using bulk transcriptome data from publicly available data bases (TCGA) and Single Cell ENergetIc metabolism by profiling Translation inhibition (SCENITH) using fresh resection material (n=4).

Results Whole transcriptome analyses of CK+ ROIs (cancer cells) identified that they clustered separately based on T cell infiltration status, indicating that CK+ cells in inflamed EACs are transcriptionally distinct from those in non-inflamed EACs. Differential gene analysis showed that antigen presentation pathway genes (HLA-A, HLA-B, HLA-C, and B2M) were upregulated in CD8-high EACs, whereas tumors with CD8-

low EACs overexpressed genes associated with attraction and M2-like polarization of macrophages, such as IDO1, CXCL5, and CSF2. Differential pathway analyses indicated that the largest difference between CD8-high and CD8-low EACs was related to lipid metabolism. CD8 high tumors show high activity of immune pathways, such as antigen presentation and IL7 signaling, whereas the CD8 low tumors were characterized by high activity of the HDL and chylomicron remodeling pathways. Pathway analysis of the CD45+ compartment identified upregulation of the PGC1a pathway in CD8 T cell low tumors, a known driver of mitochondrial biogenesis and associated with suppressive myeloid cells. The correlation between CD8 T cell status and mitochondrial metabolism was confirmed by publicly available transcriptional data from The Cancer Genome Atlas (TCGA) showing high OXPHOS and fatty acid oxidation to be associated with low cytolytic scores. Using single cell metabolic analysis (SCENITH) on fresh tumors we confirmed the presence of OXPHOS dependent myeloid cells in the TIME of CRT resistant EACs.

Conclusions Comparative analyses of tumor transcriptomes from CD8 T cell rich vs. poor areas reveal an association between downregulation of antigen presentation and chemo attraction of suppressive myeloid cells and low CD8 T cell infiltration. Furthermore, we found dysregulation of lipid metabolism in both tumor and immune cell compartments as a potential driver of immune suppression and T cell exclusion in EAC.

J. Sanders: None. **E.N. Bos:** None. **N. Hahn:** None. **I. Nijman:** None. **H.W.M. van Laarhoven:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; ORCA, Merck, Incyte, Servier, Auristone. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; Astellas, Novartis, Benecke, Daiichy-Sankyo, JAAP, Medtalks, Travel Congress Management BV. F. Consultant/Advisory Board; Modest; BMS, Dragonfly, Eli Lilly, MSD, Nordic Pharma, Servier, Amphera, Astra Zeneca, Beigene, Daiichy-Sankyo. **D.L. van der Peet:** None. **J. Van den Bossche:** None. **T. D. de Gruijl:** F. Consultant/Advisory Board; Significant; Immunicum, Lava Therapeutics. **S. Derks:** B. Research Grant (principal investigator, collaborator or consultant and pending grants as well as grants already received); Significant; Incyte. D. Speakers Bureau/Honoraria (speakers bureau, symposia, and expert witness); Modest; Servier, BMS, Benecke. F. Consultant/Advisory Board; Modest; BMS.

Plenary session 13: Cell therapies in solid oncology

13.03 EMPOWERING TCR-T CELLS FOR ADOPTIVE THERAPY FOR SOLID TUMOR THROUGH ENGINEERING WITH PD-1-BASED CHIMERIC COSTIMULATORY SWITCH PROTEINS

[1]E Noessner*, [1]C Aman, [1]AJ Fischbeck, [2]K Junker, [2]J Linxweiler. [1]*Helmholtz Zentrum München, Munich, Germany*; [2]*Saarland University, Department of Urology, Homburg/Saar, Germany*

10.1136/jitc-2024-ITOC10.10

Background Adoptive T cell therapy (ATT) has shown efficacy in the treatment of hematologic malignancies. However, in solid cancer, response rates are currently unsatisfactorily low. One of the major challenges in the treatment of solid tumors is to overcome the poor lifespan and the loss of function of tumor-reactive T cells in the tumor microenvironment (TME). We aim to overcome these hurdles by engineering tumor-reactive T cells with chimeric costimulatory switch proteins (CSPs) which should prevent PD-1/L1-mediated inhibition and simultaneous turning it into activation of costimulatory pathways leading to functional enhancement and prolonged survival.

Materials and Methods Two CSPs were generated combining the extracellular domain of PD-1 with an intracellular costimulatory domain of either CD28 or 4-1BB. T cells without CSP are controls. Primary human tumor-reactive CD8 T cells were engineered to express T cell receptors (TCRs) with HLA-A2 restricted recognition of either a tyrosinase or a renal cell carcinoma (RCC) peptide. CSP-engineered TCR-T cells and controls were in-depth characterized regarding cytokine release and cytotoxicity, phenotype, differentiation and metabolic state *in vitro* after co-culture (2D/3D-matrigel) in TME-adapted conditions (using melanoma or renal cell carcinoma (RCC) cell lines) and *in vivo* using an orthotopic human RCC mouse model. Blood, tumors and organs were analyzed for the presence and phenotype of T cells by flow cytometry.

Results *In vitro*, CSP-engineered TCR-T cells showed enhanced cytokine release and cytotoxicity. CSPs enabled maintenance of function during repeated tumor challenge and nutrient-restricted conditions. Flow cytometry identified higher Ki67, IRF4 and BATF as well as markers of metabolic fitness in CSP-TCR-T cells compared to control T cells following co-culture with PD-L1 positive tumor cells. Induced changes were dependent on tumor cells expressing PD-L1 in the context of peptide/MHC ligands. *In vivo*, using an orthotopic implant of RCC tumor cells, ATT with TCR-T cells reduced tumor volume compared to the no T cell group, and only residual tumor cells were detected after histological examination in the CSP-TCR-T cell groups. Plasma IFNγ levels at day 3 after ATT were higher for CSP-TCR-T cells compared to control T cells.

Conclusions We collected essential functional data using *in vitro* and *in vivo* models describing effects of PD-1-based CSPs on TCR-T cell phenotype and functional activity. We observed higher efficacy of PD-1-CSP-T cells in controlling orthotopic RCC xenografts, which is to our knowledge the first report of PD-1-CSP effects using non-affinity-enhanced TCR (isolated from human tumor-infiltrating T cells) and unmodified cancer cells with endogenous (not engineered) antigen/MHC and PD-L1. The results suggest that PD-1-CSPs can empower tumor antigen-reactive TCR-T cells for beneficial application in solid tumor therapy paving the way for in human clinical trials using TCR-T cells armored with CSPs.

Funded by Deutsche Krebshilfe, and ZIM/AppMic.

E. Noessner: None. **C. Aman:** None. **A.J. Fischbeck:** None. **K. Junker:** None. **J. Linxweiler:** None.

13.04 IMPROVING THE EFFICACY OF TCRTG T CELL THERAPY FOR SOLID TUMORS WITH MRNA-BASED VACCINATION

J Krueger*, J Lutz, J Ruotsalainen, R Mülfarth, R Heidenreich. *CurVac SE, Tübingen, Germany*

10.1136/jitc-2024-ITOC10.11