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

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

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P02 Microbiome and Immune System/ Immunotherapy

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

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

Materials and Methods We are currently collecting and 16S rRNA sequencing fecal biospecimen from BCP-ALL and DLBCL patients before, during and after treatment with Axicabtagene-Ciloleucel and Tisagenlecleucel at the University

Hospital of the LMU. Microbiota data are integrated into a patient-centered 'hospitalome' including onset and type of infection and of immunotoxicity, concomitant anti-infective and immunosuppressive agents as well as response to CAR T-cell therapy.

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

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

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

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

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

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

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