Materials and Methods We screened T cell activation and exhaustion markers, among others, on different tumor tissues using the MACSima™ Imaging Platform, an instrument for the highly multiplexed immunofluorescence imaging technology MICS (Multiparameter Imaging Cell Screen), enabling investigation of hundreds of markers on a single section. Moreover, flow cytometry and single-cell RNA sequencing analyses of T cells from tumor digests were performed to complement the characterization of TILs.

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

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

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

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

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P07 Cell Therapy in Haematologic Diseases

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

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

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

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

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

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

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

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

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

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

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

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

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

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P08 Combination Therapy

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

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

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