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

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

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

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

**Disclosure Information**


**P06.13**

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

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

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.

Disclosure Information E. Criado-Moronati: A. Employment (full or part-time); Significant; Miltenyi Biotec B.V. & Co. KG. A. Gosselink: A. Employment (full or part-time); Significant; Miltenyi Biotec B.V. & Co. KG. J. Kollet: A. Employment (full or part-time); Significant; Miltenyi Biotec B.V. & Co. KG. A. Dzionek: A. Employment (full or part-time); Significant; Miltenyi Biotec B.V. & Co. KG. B. Hecmskerk: A. Employment (full or part-time); Significant; Miltenyi Biotec B.V. & Co. KG.

P07 Cell Therapy in Haematologic Diseases

P07.01 CD19 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-cell) 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-cell 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 apherased 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).