

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).

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**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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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-dependant 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 GL26-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.

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**P06.14 CHARACTERIZATION OF TUMOR-INFILTRATING T CELLS BY HIGHLY MULTIPLEXED IMMUNOFLOUORESCENCE IMAGING**

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**Background** The adoptive cell transfer (ACT) of tumor-infiltrating T lymphocytes (TILs) has shown remarkable results in patients with different cancer types. The antitumor effect of this therapy is mainly attributed to a small fraction of tumor-reactive T lymphocytes (TRLs) that recognize mutated peptides as well as overexpressed self-antigens. Therefore, the enrichment and expansion of TRLs constitutes a promising immunotherapy approach. However, the specific targeting of individual mutated antigens represents a daunting challenge for widespread therapeutic application. Alternatively, we hypothesize that TRLs could be identified and enriched by a surface marker (or combination thereof) in an antigen-independent manner as a result of the chronic antigen exposure and other factors present in the tumor microenvironment (TME).