competent GL261 glioma model which recapitulates human disease and host immune barriers. We generated a library of B7-H3 CARs with different transmembrane (CD8, CD28), costimulatory (CD28, 4-1BB), and activation (ζ, mutζ) domains. We then compared their cytolytic activity, expansion, and anti-tumor activity. Results show that B7-H3 CARs with CD28 transmembrane and costimulatory domains have superior efficacy compared to CARs with CD8 and 4-1BB domains. Additionally, CARs with mutated ζ activation domain have better overall persistence. However, providing costimulation signals through CD28 or 4-1BB alone does not induce superior anti-glioma efficacy of B7-H3 CAR T-cells *in vivo*. Thus, we next investigated whether incorporating 4-1BB signaling into CD28-based CARs using *in trans* design enhances the therapeutic efficacy of B7-H3 CAR T-cells. We found that in repeat stimulation assays, surface expression of 4-1BBL enhanced expansion of B7H3 CAR T-cells at least 300-folds more than T-cells with CD28 or 4-1BB costimulatory domains alone. Additionally, 4-1BBL expression significantly enhanced the sequential killing capacity compared to CD28- or 41BB-based B7-H3 CAR T-cells. High dimensional flow cytometry analysis of GL261 tumors post CAR T-cell injection revealed unique immune clusters including dendritic cells and lymphoid predominant populations in mice treated with 4-1BBL expressing CARs. Thus, expression of 4-1BBL on CD28-based CARs reshaped the TME and enhanced persistence and anti-glioma efficacy of B7-H3 CAR T-cells. Studies examining transcriptional and epigenetic programs, and TME/CAR T-cell interactions are in progress. Results will define pathways that dictate CAR T-cell performance and will identify unique mechanisms for further improvements utilizing other members of TNF-superfamily.

IMMU-02. TARGETED INNOVATIVE ANTIBODY FRAGMENT-BASED IMMUNOTHERAPY FOR MEDULLOBLASTOMA

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Medulloblastoma (MB) is the most common malignant pediatric brain tumor accounting for ~20 % of childhood brain tumors. One third of all MB are characterized by constitutive activation of the Sonic Hedgehog (SHH)-signaling pathway. This tumor type shows overexpression of the epidermal growth-factor receptor (EGFR), which we detected in SHH-MB patient samples, transgenic SHH MB mouse models, and MB-cell lines. In contrast, non-neoplastic cells only express EGFR at low levels. Intensive radio-/chemotherapy often leaves the young patients with significant long-term burdens including problems in brain development and cognitive deficits. Thus, there is an urgent need to develop new targeted therapies that can prevent tumor recurrence without affecting healthy cells. We selected EGFR as a potential therapy target using EGFR-specific antibody fragments (scFvs) as part of immunoconjugates, namely bispecific T-cell engagers (BiTEs) and immunotoxins (ITs). Both, the EGFR-specific BiTEs and the ITs showed specific binding and cytotoxic activity in MB cells. Effector- and target-cell specificity was demonstrated via flow cytometry for the BiTEs. BiTEs and ITs selectively killed MB-tumor cells and showed pro-apoptotic effects without unspecific effects. Furthermore, preliminary results from an innovative hiPSC-based in vitro-BBB-model suggest, that the ITs are able to cross the BBB. Finally, by having a functional cloning- and expression system for the BiTEs and ITs available, target-scFvs can be easily exchanged by novel antigens or peptides to obtain additional targeted immunotherapies. Together, these results pave the way for preclinical in vivo experiments and future clinical trials in patients with SHH MB.

IMMU-03. SYNERGY BETWEEN TMZ AND INDIVIDUALIZED MULTIMODAL IMMUNOTHERAPY TO IMPROVE OVERALL SURVIVAL OF IDH1 WILD-TYPE MGMT PROMOTER-UNMETHYLATED GBM PATIENTS

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The prognosis of IDH1 wild-type MGMT promoter-unmethylated GBM patients remains poor. Addition of Temozolomide (TMZ) to first-line local treatment shifted the median overall survival (OS) from 11.8 to 12.6 months. We retrospectively analysed the value of individualized multimodal immunotherapy (IMI) to improve OS in these patients. All adults meeting the criteria and treated 06/2015-06/2021 were selected. Thirty-two patients (12f, 20m) had a median age of 47y (range 18-69) and a KPI of 70 (50-100). Extent of resection was complete (11), <complete (12) or not documented (9). Seven patients were treated with surgery/radio(chemo)therapy and subsequent IMI (Group-1); 25 patients were treated with radiochemotherapy followed by maintenance TMZ plus IMI during and after TMZ (Group-2). Age, KPI and extent of resection were not different amongst both groups. The median OS of group-1 patients was 11m (2y OS: 0%). Surprisingly the median OS of group-2 patients was 22m with 2y OS of 36% (CI95%: 16-57), which was significantly (Log-rank: p = 0.0001) different from group-1. The data suggest that addition of IMI after local therapy on its own has no relevant effect on OS in these GBM patients, similar to maintenance TMZ. However, the combination of both TMZ + IMI significantly improved OS. This finding might also have implications in the search for novel combined treatment approaches for children with malignant glioma.

IMMU-04. TRANSCRIPTIONAL ANALYSIS REVEALS DISTINCT MICROENVIRONMENTAL SUBGROUPS ACROSS PEDIATRIC NERVOUS SYSTEM TUMORS

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INTRODUCTION: Recent clinical trials of immune checkpoint inhibitors indicated 5-11% response rate in pediatric patients depending on cancer type and expression of target proteins. Currently, a systematic analysis characterizing the immune microenvironment of childhood tumors is lacking. The main objective of this study is to uncover the features of immune microenvironment in pediatric nervous system tumors (pedNST). METHODS: We compiled transcriptomes of 925 tumors from three initiatives, Therapeutically Applicable Research To Generate Effective Treatments (TARGET, n = 149), International Cancer Genome Consortium (ICGC, n = 195) and Children Brain Tumor Tissue Network (CBTN, n = 581). We analyzed the performance of immune deconvolution tools and used publicly available datasets to define immune genesets. We conducted a consensus analysis to assign genes to cell-types and identify immunological groups. RESULTS: We found wide variability in immune infiltration across and within cancer types ranging from cold tumors such as medulloblastoma (2.7% infiltrate) to infiltrated entities such as neurofibroma (22.6%). Consensus clustering revealed four distinct immune clusters. The pediatric inflamed group (10%) included MYCN non-amplified neuroblastoma and ATRT. The myeloid-predominant group (30%) showed decreased infiltration of lymphoid cells but enrichment of myeloid cell genesets. The pediatric-cold group (42%) harbored no enrichment of immune genesets and included 72% of ependymomas and 65% of medulloblastomas. The immune excluded group (18%) showed depletion of immune cell-types and included sonic-hedgehog medulloblastoma. 71% of pedNST belonged to the lymphocyte depleted or immunologically quiet clusters, indicating the cold immune microenvironment in pedNST compared to adult cancers. CONCLUSION: We report characteristics of the immune microenvironment in pedNST. We found an overall cold microenvironment with low lymphocyte infiltration in this population compared to common adult cancers. We identified ~10% of tumors harboring a relatively inflamed microenvironment. Our data uncover characteristics of immune infiltration in pediatric tumors with potential implications to guide therapy.

IMMU-05. INTEGRATIVE TRANSCRIPTOMIC ANALYSIS OF PILOCYTIC ASTROCYTOMAS REVEALS CNS REGION-ASSOCIATED CHARACTERISTICS

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