sive model, we delivered of a single dose of two million mRNA GD2-directed CAR T cells locoregionally to the pons via stereotactic injection. The mRNA GD2-directed CAR T cells resulted in no toxic deaths of the mice. In addition, a single dose of mRNA CAR T cells targeting GD2 prolonged survival of the mice by a median of six days ($p<0.05$). Ongoing studies using an indwelling catheter for repeated dosing of mRNA CAR T cells are currently underway and results expected at the time of presentation. This work will form of the basis of an mRNA CAR T cell trial targeting GD2 for patients with DIPG.

IMMU-12. PHASE I/II TRIAL OF IMMUNOTHERAPY WITH FUSIONS OF DENDRITIC CELLS AND TUMOR CELLS FOR RELAPSED OR REFRACTORY BRAIN TUMORS IN CHILDREN AND YOUNG ADULTS

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BACKGROUND/OBJECTIVES: Relapsed or refractory brain tumors in childhood continue to have a dismal prognosis in spite of intensive multidisciplinary treatment. Cancer immunotherapy is newly developed to be expected as next promising treatment for highly aggressive pediatric cancer. This trial was designed to evaluate the safety and effectiveness of an immunotherapy with fusions of dendritic cells (DCs) and tumor cells in patients with malignant brain tumors. METHODS: Patients with histopathologically confirmed malignant and recurrent/refractory brain tumor were eligible for this immunotherapy trial. Autologous cultured tumor cells obtained from surgical specimens were fused with autologous DCs using polyethylene glycol. The fusion cells (FC) were inoculated intradermally in the cervical region and repeated 3–10 times in each 28–84 days cycle. Treatment-related toxicity, progression-free survival (PFS), and overall survival (OS) were evaluated. RESULTS: Six patients were enrolled, three with high grade glioma and three with ependymoma. Median age at first course of immunotherapy was 10 years (range 8–25 years) and median follow-up time from the first course of immunotherapy was 13.5 months (range 3–33 months). All patients with immunotherapy were well tolerated to this treatment with no adverse events except local erythema in injected site. Median progression free survival and overall survival were 18 months and 18.5 months, respectively. CONCLUSIONS: FC immunotherapy with autologous DCs and tumor cells for brain tumor in children and young adults were extremely well tolerated and showed encouraging responses in this series. Further phase II study of FC immunotherapy is planned to improve survival and reduce treatment related morbidity.

IMMU-13. DUAL IGF1R/IR INHIBITOR IN COMBINATION WITH GD2-CAR T-CELLS AS A POTENT THERAPEUTIC STRATEGY FOR H3K27M-MUTANT DIFFUSE MIDLINE GLIOMAS

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Diffuse midline gliomas (DMG) are aggressive paediatric brain tumors for which there is no effective treatment. Recent pre-clinical studies suggest that adoptive transfer of chimeric antigen receptor (CAR) T-cells targeting the disialoganglioside antigen GD2 (GD2-CAR) has a significant therapeutic potential for H3K27M-mutant DMG. Still, some tumor cells resist to treatment suggesting that a multimodal approach may be necessary to treat more efficiently the disease. Our aim was to identify chemical compounds that, in combination with CAR T-cells, would enhance anti-tumor efficacy. After having confirmed the GD2 expression in tissue samples and patient-derived H3K27M-mutant DMG cells, we developed a high throughput cell-based assay to screen 40 kinase inhibitors in combination with T-cells expressing the GD2-CAR.CD28.4-1BB.z construct. The screening led to the identification of the dual IGF1R/IR antagonists, BMS-754807 and linsitinib, which, in combination with GD2-CAR T-cells, improved antitumor activity by 25% ($p<0.0001$) and 20% ($p<0.0001$) respectively, compared to GD2-CAR T-cells alone. The two compounds inhibited tumor cell proliferation through IGF1R/IR dependent mechanisms at a concentration which did not affect CAR T-cell expansion. Linsitinib, but not BMS-754807, decreased GD2-CAR T-cells exhaustion and increased their memory profile. Furthermore, linsitinib attenuated the expression of 10 out of 71 DMG genes involved in immunomodulation (*e.g.* IL33, VEGFC, STAT5A) and regulated upon tumor/CAR T-cells co-culture. Finally, we confirmed the anti-tumor activity of the new linsitinib/GD2-CAR T-cells combination strategy in a DMG H3K27M-mutant 3D culture model. Our work supports the development of IGF1R/IR inhibitors to be used in combination with GD2-CAR T-cells for H3K27M-mutant DMG therapy.

IMMU-14. IMMUNE CHECKPOINT INHIBITOR THERAPY FOR TREATMENT OF SYNCHRONOUS CANCERS IN PAEDIATRIC PATIENTS WITH CONSTITUTIONAL MISMATCH REPAIR DEFICIENCY

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Constitutional mismatch repair deficiency (CMMRD) is an autosomal recessive condition in which affected patients carry biallelic germline mutations in the MMR genes. This highly penetrant syndrome results in nearly universal development of malignant neoplasms at a young age, most commonly pediatric brain tumors. Importantly, in addition to brain tumors, patients frequently develop multiple metachronous or even synchronous tumors making it impossible to treat these cancers with current chemotherapeutic approaches due to the complexity of different chemoradiation regimens required, resulting in excess toxicity and lack of efficacy. We first, assessed the metachronous (defined here as serial tumors diagnosed >1 year apart or after completion of definitive treatment for the initial tumor) or synchronous cancers (defined here as tumors diagnosed within a year of each other or during the definitive treatment for the initial tumor) in all patients within the consortium. Strikingly, 47% developed synchronous and/or metachronous cancers leading to patient demise. Molecular analysis revealed that all synchronous tumors (n=26) harbored a hypermutational burden accompanied by high genomic microsatellite instability and the relevant signatures. We therefore treated two patients with glioblastomas who had synchronous solid tumors with checkpoint inhibitors. In both patients, objective tumor response was associated with clinical benefit and prolonged survival. Biomarker analysis revealed increased tumor mutational burden, microsatellite instability and immune cell infiltration. These cases highlight the role of universal, mechanism based and tumor-agnostic approach to treat patients with brain tumors with additional synchronous cancers in the setting of cancer predisposition.

IMMU-15. PROTEOGENOMIC DISCOVERY OF NOVEL TUMOR PEPTIDES AS NEOANTIGENS FOR PERSONALIZED T CELL IMMUNOTHERAPY IN MEDULLOBLASTOMA

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T cell immunotherapies are promising new tools to combat high-risk subgroups of medulloblastoma without increasing the late effects burden. The ideal target antigen of an effective antitumor T-cell response is abundantly expressed by tumor cells but not by normal tissues, in order to limit off-target effects. Tumors translate a host of highly novel transcripts that are the result of aberrations in tumor DNA and the unmasking of alternative or novel exons. We developed a novel proteogenomic approach to identify tumor-restricted peptides and used them to select and expand T cells capable of mounting a tumor-specific cytotoxic immune response. Using RNA-seq and WGS data, we created personalized custom searchable databases containing predicted novel proteins from somatic mutations, novel junctions and fusion transcripts from 56 medulloblastoma tumors. By searching these databases with raw mass spectrometry data from paired medulloblastoma tumors, we identified tens of neoantigen peptides arising from the translation of tumor-specific transcripts; novel isoforms being the predominant source. We tested these peptides for their ability to select and expand autologous polyclonal populations of T cells and tested the immunogenicity of each individual peptide. Flow cytometry revealed populations of CD4+ and CD8+ cells with an activation profile marked by IFN-γ and TNF-α. Immunosuppressive marker profiles were also characterized. Using cytotoxicity assays, we demonstrated that tumor specific T cells can eliminate neoantigen bearing tumor cells. Thus, proteogenomics can identify immunogenic tumor