patients. NIVO and IPI first dose trough concentrations were lower in youngest and lowest-weight patients. Baseline tumor PD-L1 expression was not associated with survival. Tumor mutational burden was high in 1 patient (NIVO+IPI) with HGG (OS=11.0mos). CONCLUSIONS: NIVO±IPI demonstrated no clinical benefit in pediatric patients with high-grade CNS malignancies, consistent with available historical data. The safety profiles were manageable.

IMMU-09. INTERIM ANALYSIS FROM BRAINCHILD-03: SEATTLE CHILDREN'S LOCOREGIONAL B7-H3 CAR T CELL TRIAL FOR CHILDREN WITH RECURRENT CENTRAL NERVOUS SYSTEM TUMORS AND DIPG

Nicholas Vitanza[1,2], Juliane Gust[3,4], Ashley Wilson[5], Wenjun Huang[5], Dickson Chen[6], Michael Meechan[1], Matt Biery[1], Carrie Myers[1], Sophie Tahiri[1], Erin Crotty[1,2], Sarah Leary[1,2], Bonnie Cole[7,8], Samuel Browd[9], Jason Hauptman[9], Amy Lee[9], Catherine Albert[1,2], Navin Pinto[1,2], Rimas Orentas[1,2], Rebecca Gardner[1,2], Michael Jensen[5], Julie Park[1,2]; [1]The Ben Towne Center for Childhood Cancer Research, Seattle Children's Research Institute, Seattle, WA, USA. [2]Division of Pediatric Hematology, Oncology, Bone Marrow Transplant, and Cellular Therapy, Department of Pediatrics, University of Washington, Seattle, WA, USA. [3]Department of Neurology, University of Washington, Seattle, WA, USA. [4]Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, WA, USA. [5]Seattle Children's Therapeutics, Seattle, WA, USA. [6]Center for Clinical and Translational Research, Seattle Children's Research Institute, Seattle, WA, USA. [7]Department of Laboratories, Seattle Children's Hospital, Seattle, WA, USA. [8]Department of Laboratory Medicine and Pathology, University of Washington School of Medicine,, Seattle, WA, USA. [9]Division of Neurosurgery, Department of Neurological Surgery, Seattle Children's Hospital, Seattle, WA, USA

BrainChild-03 is a phase 1 clinical trial delivering repeated locoregional 2nd generation B7-H3 CAR T cells with 4-1BB co-stimulation to children with central nervous system (CNS) tumors without lymphodepleting chemotherapy. The primary endpoints are feasibility and safety, with secondary endpoints of disease response and correlatives of CAR T cell activity. There are 3 arms: (A) – weekly delivery into the tumor cavity, (B) – weekly delivery into the lateral ventricle for metastatic disease, (C) – biweekly delivery into the lateral ventricle for diffuse intrinsic pontine glioma (DIPG). In total, 23/24 (96%) enrolled patients have had successful CAR T manufacturing. 16/24 patients are evaluable and have received a total of 141 intracranial CAR T cell doses. Unevaluable patients include 5 never treated and 3 who progressed prior to receiving the minimum doses to become evaluable. The most common adverse events have been headache (16/16, 100%), nausea/vomiting (12/16, 75%), and fever (10/16, 63%). There has been 1 DLT for an intratumoral hemorrhage and no cytokine release syndrome (CRS). 7 evaluable patients with DIPG (Arm C) have received a cumulative 50 infusions. 5/7 DIPG patients enrolled after progression and have a median survival of 246.5 days post-initial CAR T cell infusion, with 4/5 still alive. The 2 DIPG patients enrolled prior to progression had radiographic improvement, including 1 with improvement of a cranial nerve 6 palsy who self-withdrew from protocol therapy after 18 infusions over 12 months and 1 still on protocol therapy after 11 infusions over 6 months. DIPG patients have had increased CSF levels of proinflammatory mediators (e.g. CXCL10, CCL2, IFNg, GM-CSF, IL-12) without systemic cytokine changes. 5/7 DIPG patients had detectable CAR T cells in their CSF post-infusion. Ultimately, the preliminary experience suggests locoregional delivery of B7-H3 CAR T cells may be feasible and tolerable in children with CNS tumors, including DIPG.

IMMU-10. TUMOR ASSOCIATED MYELOID CELLS DRIVE THE IMMUNOBIOLOGY OF HIGH RISK PEDIATRIC EPENDYMOMA

Andrea Griesinger[1,2], Kent Riemondy[1], Nithyashri Eswaran[1], Andrew Donson[1,2], Nicholas Willard[1,2], Simon Paine[3], Georgia Bowes[3], John Rheaume[4], Rebecca Chapman[3], Judith Ramage[3], Andrew Jackson[3], Richard Grundy[3], Nicholas Foreman[1,2], Timothy Ritzmann[3]; [1]University of Colorado Denver, Aurora, CO, USA. [2]Children's Hospital Colorado, Aurora, CO, USA. [3]University of Nottingham, Nottingham, United Kingdom. [4]Isoplexis, Branchford, CT, USA

Molecular profiling of pediatric ependymoma (EPN) has previously identified discrete neoplastic subpopulations, of which Mesenchymal EPN Cells (MEC) characterize Posterior Fossa A tumors (PFA). MECs are associated with tumor immunosuppression. Here we further characterize the EPN immune environment using single-cell sequencing, spatial phenotyping and cytokine analyses to better define infiltrating myeloid subpopulations. We hypothesize that neoplastic and myeloid cells interact to propagate an immune suppressive environment conferring resistance to traditional therapies. We delineated myeloid cell subpopulations from single-cell RNA-seq of 26 pediatric EPNs and validated them through deconvolution of bulk gene expression profiling (n=299). To define subpopulation spatial distribution, we interrogated a range of tumor and myeloid markers using multiplex immunofluorescence (mIF). Finally, using single-cell cytokine analyses, we gained further insight into myeloid subpopulation function. Eight distinct myeloid subpopulations were identified, relating to macrophages, microglia and monocytes. A subpopulation of cells with wound healing ontologies and characterized by TREM1 expression, demonstrated features of myeloid derived suppressor cells, including IL6/STAT3 pathway activation. We called these hypoxia-M. Like MEC neoplastic cells, hypoxia-M was associated specifically with PFA1 subgroup EPN in both single-cell and bulk tumor gene expression profiling ($p<0.001$). Additionally, the presence of MEC and hypoxia-M correlated strongly in gene expression ($r2=0.92$, $p<0.001$) and IHC analyses, where they co-located to borders between necrosis, blood vessels and viable tumor. Analysis using mIF (n=54) confirmed MEC/hypoxia-M co-location and highlighted that all types of immune cell corralled in significant numbers around areas of vasculature and necrosis. Single cell cytokine analyses demonstrated that hypoxia-M secrete IL-8 which, we hypothesize, amplify the pro-tumor phenotype in PFA1 tumor microenvironment. EPN is characterized by discrete myeloid cell subpopulations which contribute to the tumor microenvironment. Treatment strategies must focus on modifying this pro-tumor, immunosuppressive microenvironment to deliver more effective treatment for childhood ependymoma.

IMMU-11. EVALUATION OF CAR-T CELLS TARGETING CD276 IN MEDULLOBLASTOMA

Patricia Benites Goncalves da Silva[1,2], Laura Sieber[1,2], Norman Mack[1,2], Daniel Atar[3], Lena M. Kutscher[1,2], Patrick Schlegel[4], Martin Ebinger[3], Daisuke Kawauchi[5], Christian Seitz[3], Stefan M. Pfister[1,2]; [1]Hopp Kindertumorzentrum Heidelberg (KiTZ), Heidelberg, Germany. [2]Division of Pediatric Neurooncology, German Cancer Consortium (DKTK) and German Cancer Research Center (DKFZ), Heidelberg, Germany. [3]Clinic for Pediatrics and Adolescent Medicin, General paediatrics, hematology and oncology, Tübingen University Hospital, Tübingen, Germany. [4]Cellular Cancer Therapeutics, School of Medical Sciences / Medical Sciences Administration, Faculty of Medicine and Health (FMH), University of Sydney, Sydney, Australia. [5]Department of Biochemistry and Cellular Biology, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan

Brain tumors are the most frequent category of solid tumors in children and have the highest mortality rate among all pediatric cancers. Although diagnosis and treatment have improved prognosis over the past decades for some childhood brain tumors, others remain lethal and current treatments are highly toxic to the developing brain, resulting in severe sequelae and considerably affecting the patient's quality of life. Thus, new therapeutic options with reduced secondary effects are urgently needed. From this perspective, immunotherapies have gained a lot of attention due to their effectiveness in targeting tumor cells specifically. Chimeric-antigen receptor (CAR) T-cells recognize the target antigen on the surface of. CD276 is an immune checkpoint molecule that is expressed in a variety of solid tumor entities, including pediatric brain tumors. We analyzed the CD276 expression in our Patient-Derived-Xenograft (PDX) biobank of brain tumors and found that CD276 is ubiquitously expressed (ATRT, MB, EPN, GBM, ETMR, etc). Flow cytometry of MB PDX (n=4) confirmed CD276 expression of 97-99% of tumor cells, indicating that CD276 might be a good antigen target for CAR-T cell therapy of MB_{G3} and MB_{SHH}. We found that second generation CAR-T cells targeting CD276 antigen significantly decreased tumor burden of the most aggressive MB subgroups (G3 and SHH-TP53mut PDX models) in NSG mice. We further treated NSG mice carrying a high tumor burden of the aggressive SHH-TP53mut PDX BT084 with second (CD28) and third generation (CD28-41BB) CD276-CAR-T cells. While both 2nd and 3rd generation improved the survival rates compared with CD19-CAR-T control cells, we found no difference in survival between the CD276 CAR-T generations, with no severe secondary effect during treatment. In conclusion, CD276 is a good antigen target for medulloblastoma, and warrants further evaluation for the treatment of medulloblastoma patients at relapse or as a maintenance therapy after standard treatment.

IMMU-12. EXPLORING AND MODULATING THE TUMOUR IMMUNE MICROENVIRONMENT TO FACILITATE THE SELECTION OF IMMUNOTHERAPIES FOR PAEDIATRIC-TYPE DIFFUSE HIGH-GRADE GLIOMA

Julia Cockle[1,2], Lynn Bjerke[1], Alan Mackay[1], Yura Grabovska[1], Anna Burford[1], Valeria Molinari[1], Rita Pereira[1], Jessica Boult[1], Simon Robinson[1], Diana Martins Carvalho[1], Matthew Clarke[1], Ian Titley[1], Erika Yara[1], Karin Straathof[3], Erik Wennerberg[1], Oren Becher[4], Maria Castro[5], Alan Melcher[1], Chris Jones[1]; [1]Institute of Cancer Research, London, United Kingdom. [2]The Royal Marsden NHS Foundation Trust, Surrey, United Kingdom. [3]University College London, Great Ormond Street Institute of Child Health, London, United Kingdom. [4]Mount Sinai Kravis Children's Hospital, New York, USA. [5]University of Michigan Medical School, Michigan, USA

Immune cells have the potential to selectively eradicate high-risk brain tumours such as paediatric-type diffuse high-grade glioma (PDHGG). We