therapy (ipilimumab/nivolumab); two patients received monotherapy with either nivolumab or pembrolizumab. Median time from initial diagnosis-to-treatment was 8 months (range 0.8–156). Ten patients received radiation therapy (RT) prior to immunotherapy, with one receiving concurrent RT. Median duration of treatment was 6.1 months (range:1–19). Therapy was discontinued in nine patients: seven due to disease progression and two due to adverse events (colitis, transaminitis). Other pertinent toxicities included type 1 diabetes, hypothyroidism and skin toxicity. Based on iRANO criteria, best responses included partial (n=4), stable (n=6) and progressive disease (n=1). Durable response (>12months) was noted in two patients (HGG and progressive NGGCT). CONCLUSION: Immune checkpoint inhibition appears to have clinical benefit and is relatively well tolerated in this cohort of patients. Results from recently completed prospective clinical trials will be critical to inform clinical decisions.

IMMU-02. CHIMERIC ANTIGEN RECEPTOR (CAR) T CELL NEUROTOXICITY CORRELATES WITH PRETREATMENT AND ACUTE CSF NEUROFILAMENT LIGHT CHAIN (NFL) LEVELS

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OBJECTIVE: Immunotherapy for hematologic malignancies with CD19-directed CAR T cells is complicated by neurotoxicity in approximately 40% of patients. We have previously reported evidence of glial injury in pediatric patients with CAR T neurotoxicity by elevated CSF levels of GFAP and S100b. We now hypothesize that NFL is also a useful biomarker of neuronal injury related to abnormal blood-brain-barrier and glial function. METHODS: We used the Mesoscale Discovery platform to measure CSF and serum NFL levels in a consecutive cohort of 43 pediatric patients with B cell ALL who received CD19-directed CAR T cells. In addition, we will present expansion cohort measurements of NFL and GFAP (N=95). RESULTS: CSF NFL levels prior to CAR T cell infusion positively correlated with the risk of subsequently developing severe neurotoxicity (no neurotoxicity, median 275pg/mL, mild 378pg/mL, severe 951pg/mL, P=0.0182 for severe vs none, P=0.0458 for severe vs mild). During neurotoxicity, mean CSF NFL levels increased to 1179pg/mL (mild neurotoxicity, P=0.0338) and 1345 pg/mL (severe neurotoxicity, P=0.0148), respectively. In serum, pretreatment NFL levels were highly abnormal in many patients (median 368pg/mL, range 10–56,321pg/mL; healthy control median 4pg/mL, range 1–7.5pg/mL). However, there was no correlation with neurotoxicity, history of CNS radiation, peripheral neuropathy, stem cell transplant, or number of prior chemotherapies. Day 7 serum NFL levels did not change significantly (median 439pg/mL, range 5–17,439pg/mL, P=0.3254). CONCLUSION: We conclude that CSF NFL is promising biomarker of CAR T neurotoxicity risk and severity. The abnormal baseline serum NFL concentrations remain unexplained and require further study.

IMMU-03. UPDATES ON BRAINCHILD-01, -02, AND -03: PHASE 1 LOCOREGIONAL CAR T CELL TRIALS TARGETING HER2, EGFR, AND B7-H3 FOR CHILDREN WITH RECURRENT CNS TUMORS AND DIPG

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We report preliminary results of three Phase 1 trials of repetitively dosed locoregional CAR T cells for children with recurrent/refractory CNS tumors, targeting HER2 (BrainChild-01), EGFR (BrainChild-02), and B7-H3 (BrainChild-03). Cells are delivered into the tumor cavity (Arm A) or ventricular system (Arm B and BrainChild-03's DIPG-specific Arm C). Primary endpoints are feasibility and safety. Successful CAR T cell manufacture occurred in 2/2 subjects (BrainChild-01) and 2/3 (BrainChild-02). All subjects tolerated intra-patient dose escalation from $1x10^7$ to $2.5x10^7$ cells/dose without DLTs. Two subjects were evaluable on BrainChild-01 (S-001: glioblastoma, Arm A, survival 173 days post-first infusion, received 6 infusions; S-002: ependymoma, Arm B, survival 111 days, 9 infusions). One subject was evaluable on BrainChild-02 (glioblastoma, Arm A, withdrew from trial at 49 days, 5 infusions). One enrolled patient on BrainChild-03 has not begun treatment. None of the subjects developed new neurologic toxicities, although transient worsening of baseline tumor-related signs and symptoms were seen. Secondary endpoints are efficacy and disease response. No objective radiographic responses have been observed. Both BrainChild-01 subjects had transient systemic CRP elevations following infusions (S-001: peak of 3.9 post Course 1 Week 1; S-002: peak of 2.3 post Course 2 Week 1), possibly indicating an inflammatory response. Both subjects had post-infusion CSF cytokine elevations (CXCL10, GCSF, GM-CSF, IFNa2, IFNg, IL-10, IL12-p40, IL12-p70, IL-15, IL-1a, IL-3, IL-6, IL-7, TNFa, VEGF) without concurrent systemic changes. In summary, we provide preliminary evidence of safety and feasibility of intracranial delivery of CAR T cells for pediatric CNS tumors.

IMMU-05. B7-H3-SPECIFIC CAR T CELLS HAVE POTENT ANTI-TUMOR ACTIVITY IN THE GL261 IMMUNE-COMPETENT MURINE BRAIN TUMOR MODEL

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BACKGROUND: We and others have identified B7-H3 (CD276) as a promising target for CAR-based immunotherapies for pediatric brain tumors. So far, B7-H3-CAR T cells have only been studied in xenograft models for brain tumors, which do not recapitulate the immunosuppressive tumor microenvironment (TME). To overcome this obstacle, we decided to adapt the immune-competent GL261 murine glioma model which mimics human disease and host immune barriers. METHODS: To evaluate the safety and efficacy of antigen-specific CAR T cells, murine B7-H3-CAR T cells were generated using retroviral particles encoding 2nd generation B7-H3-specific CD28.z CAR. Expansion, persistence, and anti-tumor activity were evaluated *in vitro* and *in vivo*. Components of the brain TME were then evaluated using flow cytometry and immunostaining. RESULTS: B7-H3-CAR T cells only killed B7-H3+ tumor cells, secreted significant levels of IFNγ and IL-2 in an antigen-dependent manner and expanded an average of 33-fold in repeat stimulation assay with B7-H3+ tumor cells in contrast to control CAR T cells. *In vivo*, intratumoral injection of B7-H3-CAR T cells into orthotopic GL261 glioma induced complete regression in 60% of treated mice. Preliminary studies show numerous infiltration of suppressive tumor-associated macrophages within the tumor and its periphery. CONCLUSIONS: In summary, we successfully generated murine B7-H3-CAR T cells and have demonstrated that these cells have potent anti-tumor activity in the immune-competent GL261 glioma model. However, it is likely that the tumor-associated macrophages are mediating immunosuppressive effects on B7-H3-CAR T cells. Therefore, studies focusing on TME/CAR T cell interactions are in progress.

IMMU-06. T-CELL IMMUNOTHERAPY FOR PEDIATRIC BRAIN TUMORS: DIVERSITY IN CELL SURFACE ANTIGEN AND HLA EXPRESSION NECESSITATES A MULTI-PRONGED APPROACH

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Cell surface or intracellular antigens expressed in pediatric brain tumors are potential targets for chimeric antigen receptor (CAR) or ab (T-cell receptor) TCR T-cell immunotherapy. At present it remains unknown what cell surface antigens are suitable CAR targets for pediatric brain tumors; in addition, cell surface expression of HLA class I, a molecule critical for ab TCR T-cell recognition, has not been systemically studied in these tumors. Therefore, we set out to assess expression of five CAR targets (IL13Ra2, HER2, EphA2, B7-H3, GD2) and HLA class I. We established and validated a flow cytometry-based method to profile CAR targets and HLA class I expression from pediatric patient-derived xenograft (PDX) samples. To date, we profiled 53 PDX samples, including medulloblastoma, HGG, DIPG, ATRT, and ependymoma. We found that antigen expression has high intra- and inter-PDX sample variability with B7-H3 and IL13Ra2 being most consistently expressed. We confirmed these findings using conventional IHC for B7-H3 with PDX samples and patient tissue microarrays. HLA class I was present on the cell surface of HGGs and DIPGs, however significantly down-regulated in 26 out of 36 other brain tumor types. Finally, matched fresh tissue and PDX sample analysis revealed that cells derived from PDX models are indeed representative of fresh tissue. Our results indicate that more than one antigen needs to be targeted to achieve a more complete tumor clearance. In addition, variable expression