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Diffuse intrinsic pontine glioma (DIPG) is the leading cause of brain tumor-related death in children. It is characterised for having a non-inflammatory microenvironment and be immunologically inert. Therefore, strategies aiming to break the microenvironment status-quo in this disease could provide therapeutic benefit. The complement system promotes tumor progression due to the continuous production of anaphylatoxins leading to the infiltration of myeloid cells, which express high levels of complement receptors (C3aR and C5aR1). We have in silico data showing the high expression of C5aR1 in DIPGs. Thus, we wanted to assess first whether complement C5aR1 could constitute an actionable target, and second whether combining C5aR1 inhibitors with oncolytic virus could result in a superior antitumor immune response than either agent alone in DIPG. In this study, we used two different peptide inhibitors of C5aR1, PMX53 and PMX205 combined with the virus Delta-24-ACT (an oncolytic virus armed with 4-1BBL). We performed *in vivo* studies to evaluate the efficacy of this combination in immunocompetent DIPG models. Our data showed that the combination Delta-24-ACT/PMX53 significantly extended the median survival of the animals when compared with either agent alone, and led to long-term survivors that generated immune memory. The combination treatment modulated the tumor microenvironment promoting an increase in lymphocytes, mainly CD8+ cells presenting an active phenotype, and a reduction in C5aR1 expression in the myeloid compartment. We are currently evaluating *in vivo* whether PMX205, which has an improved ability to cross the blood brain barrier, leads to better therapeutic response. In summary, the combination of Delta-24-ACT with a C5aR1 inhibitor showed the capacity to shake the DIPG tumor microenvironment and unleashed an antitumor immune response. These data underscore the possibilities to combine oncolytic virus with targets of the tumor microenvironment to improve their therapeutic benefit in DIPGs.

IMMU-10. USE OF A SINGLE PEPTIDE CHECKPOINT INHIBITOR FOR TREATMENT OF CENTRAL NERVOUS SYSTEM TUMORS

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Cancer immunotherapy has revolutionized clinical management of malignancies by generating long-term, durable control of tumors. Unfortunately, these therapies often cause serious immune-related adverse events. In addition, only a small percentage of solid tumors respond to these therapies and there is little efficacy in CNS tumors. Our research is focused on the CD200 immune checkpoint, which modulates the immune system through the inhibitory receptor (CD200R1) and activation receptors (CD200AR). We demonstrated that targeting the CD200AR with a checkpoint peptide ligand (CD200AR-L) activates the immune system and renders it impervious to the inhibitory effects of CD200. In a pre-clinical canine spontaneous high-grade glioma trial, CD200AR-L, with autologous tumor lysate vaccination, resulted in a 20% two-year progression-free survival; no toxicities or adverse effects were observed. We suggest this result was due to the ability of the CD200AR-L to modulate multiple immune checkpoints. During the characterization of the CD200AR-L, we discovered signaling molecules are shared by the CD200 and PD-1/PD-L1 checkpoint pathways, suggesting these immune checkpoints are connected. Our preliminary studies demonstrated that the inhibitory CD200R1 and PD-1 mediate immune checkpoint signaling activities through the SHIP1/2. Moreover, CD200AR-L overpowers the suppressive effects of CD200 and PD-L1, which are both shed by tumors, by downregulating the inhibitory CD200R1 and PD-1 on both antigen-presenting cells (APC) and T-cells. In addition, CD200AR-L downregulates PD-1 on APCs and inhibits the upregulation of PD-L1 and CTLA4. These studies led to the discovery that the novel peptide modulates the CD200, PD-1/PD-L1 and CTLA-4 pathways, providing the basis for the translatable development of a CD200-directed peptide for clinical use against multiple tumors including gliomas. These studies led to FDA approval of this peptide for the first in human phase I single center, open-label, dose-escalation clinical trial (NCT04642937) in adult and pediatric trial for children with recurrent malignant brain tumors.

IMMU-11. CLINICAL UPDATES AND CORRELATIVE FINDINGS FROM THE FIRST PATIENT WITH DIPG TREATED WITH INTRACRANIAL CAR T CELLS

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We report preliminary data for the first subject with diffuse intrinsic pontine glioma (DIPG) treated with intracranial CAR T cells. BrainChild-03 (NCT04185038) is a phase 1 trial of repetitively-dosed locoregional B7-H3-specific CAR T cells for children with recurrent/refractory central nervous system (CNS) tumors or DIPG. DIPG patients enroll on Arm C, on which B7H3CARs are delivered into the ventricular system via a CNS reservoir catheter. This study does not use lymphodepletion. Primary endpoints are feasibility and safety, with second endpoints of disease response. This 18-year-old female (BrainChild-03 S005) with radiographically-classic DIPG and biopsy-confirmed *H3 K27M* mutation enrolled on Arm C after progression 552 days from diagnosis following focal radiation and temozolomide, irinotecan, and bevacizumab. Apheresis and manufacturing produced $4.2x10^9$ second-generation B7H3CARs with a methotrexate-resistant human DHFR mutein (huDHFR^{FS}; L22F,F31S) in a single transcript in combination with the B7-H3-specific CAR and EGFRt, each separated by a T2A linker, allowing methotrexate selection and enrichment. At time of submission, she has received 10 every-other-week outpatient infusions of $1x10^7$ B7H3CARs (first dose on October 2, 2020). She has had no DLTs, but has experienced grade 2 fever and grade 2–3 headache peaking ~12–48 hours after each infusion. Following the 8th CAR T cell infusion, she experienced increased focal weakness and dysarthria at ~72 hours with resolution after 48 hours. She has not experienced cytokine release syndrome (CRS). She has stable disease 138 days post-initial CAR T cell infusion. Frequently collected correlative studies have detected viable B7H3CARs in the CSF post-infusion via flow cytometry. CSF cytokine analysis has revealed elevations of CXCL10, GM-CSF, and G-CSF following B7H3CAR infusions, without correlation in the serum. A second evaluable subject with DIPG has also received 4 locoregional doses of $1x10^7$ B7H3CARs without a DLT. She also has stable disease and detectable viable B7H3CARs in the CSF.

IMMU-13. CUSTOMIZABLE MULTI-LAMELLAR RNA-NANOPARTICLES FOR PEDIATRIC GLIOMA

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Background: Since the preponderance of pediatric gliomas are mutationally 'bland,' immune checkpoint inhibitors are unlikely to mediate therapeutic benefit. Alternately, immunologic response can be induced de novo against pediatric gliomas with mRNA cancer vaccines. Messenger RNA represents a paradigm shift in vaccinology (i.e. COVID-19) given its flexibility, commercialization, and propensity to confer rapid protection with only a single vaccine. Objective: We sought to develop a new mRNA platform with an optimized backbone for insertion of both personalized and/or "off the shelf' (i.e. H3K27M) transcripts for rapid induction of anti-tumor activity against pediatric gliomas. Approach: We synthesized an mRNA backbone with optimized 5' and 3' UTRs for delivery of gene transcripts pertinent to pediatric brain tumors using a lipid-nanoparticle (NP) delivery vehicle. This vaccine utilizes a novel engineering design that layers tumor derived mRNA into a lipid-nanoparticle (NP) "onion-like" or multi-lamellar package. Results: We demonstrate immunogenicity of RNA-NPs delivering either personalized glioma mRNA or H3K27M mRNA. RNA-NPs localize to myeloid cells in murine KR158b brain tumors and activate dendritic cells that supplant regulatory intratumoral myeloid populations inducing antigen-recall response with long-term survivor benefit. Our optimized mRNA backbone yielded significantly improved anti-tumor efficacy compared with commercial backbones. We have shown this approach can be refined for co-delivery of immunomodulatory RNAs (i.e. GM-CSF) and/or delivery of siRNAs targeting immunoregulatory axes (PD-L1) in murine brain tumors (GL261). We have since established safety of RNA-NPs in acute/chronic murine GLP toxicity studies without cross-reactivity to normal-brain, and launched a large-animal canine brain tumor trial which demonstrated RNA-NPs to be feasible, safe and immunologically active. Conclusion: RNA-NPs reprogram the brain tumor microenvironment while inducing a glioma-specific immune response. We have since received FDA-IND approval for first-in-human trials (IND#BB-19304) in pediatric patients with high-grade gliomas (PNOC020 study, NCT04573140).