(TAMs). We tested whether systemically administered resiquimod modulated TAMs in a genetic Sonic hedgehog (SHH) medulloblastoma model, and whether this modulation would be therapeutically beneficial. We generated medulloblastoma by crossing hGFAP-Cre/SmoM2 (G-Smo) mice. The resulting hGFAP-Cre/SmoM2 (G-Smo) mice developed medulloblastoma with 100% frequency and showed a median survival of 14.5 days (n=12). Treatment with 3 doses of resiquimod at postnatal days 10, 12 and 14 reduced tumor size and increased median survival (152 days; p=0.00358). Cellular studies showed that resiquimod altered TAM phenotype, rapidly inducing expression of the inflammatory marker VCAM1, and more slowly increasing TAM populations. Responses to the 3-dose regimen were ultimately limited by recurrence and all mice eventually died of tumor progression. Continued resiquimod therapy with every other day dosing was less effective than the 3-dose regimen, suggesting that TAM responses to resiquimod are dynamic and change with prolonged exposure. Our data show that innate immunity, mediated by TAMs and stimulated by TLR-7/8 agonist, creates a significant anti-tumor effect in medulloblastoma. The common expression of TLR-7/8 on TAMs in patient-derived medulloblastoma samples and in the mouse model suggests that resiquimod may produce similar anti-medulloblastoma effects in humans. Further studies are needed to define the mechanism of the anti-tumor effect in detail, to determine if resiquimod can combine effectively with additional adjuvant therapies to produce curative effects.

**IMMU-06. DELTA-24-RGD EXPRESSING POSITIVE IMMUNE MODULATORS SHOW ANTI-DIPG EFFECT AND INCREASE TUMOR IMMUNE INFILTRATION**

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Objective: To determine the clinical variances between strokes and stroke mimics in a pediatric immunocompromised population that consists predominantly of children with central nervous system (CNS) and non-CNS malignancies. A history of solid organ transplantation was excluded. Methods: We performed a retrospective cohort analysis of stroke alert activations in patients with high-grade gliomas, low-grade gliomas, atypical teratoid rhabdoid tumors, rare CNS tumors, B-cell acute lymphoblastic leukemia, T-cell acute lymphoblastic leukemia, osteosarcoma, and solid organ transplants at St. Louis Children’s Hospital between February 2013 and September 2019. We categorized final diagnoses as strokes or stroke mimics. We classified diagnoses as a neurologic emergency when there was suspicion of stroke during patient management. Results: Out of 217 stroke alerts, 31 alerts occurred for 28 patients meeting inclusion criteria. All final diagnoses constituted neurologic emergencies, including: stroke (39%), chemotherapy-related toxicity (23%), and seizures/postictal status (30%). Conclusions: 2021

**IMMU-08. MICROENVIRONMENT MODULATION BY TIM-3 BLOCKADE IMPROVES THE OUTCOME OF PRECLINICAL DIPG MODELS**

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Diffuse Intrinsic Pontine Gliomas (DIPG) are aggressive pediatric brain tumors that arise in the pons of children, being the leading cause of pediatric brainstem tumors and in brains of treated mice. Moreover, the immune infiltrated showed a functional profile showing increased survival of mice bearing orthotopic DIPG murine tumors, leading to long-term survivors (50%) and showed a significant increase in B, NK and CD8+ cells. In vivo, the three virus were able to infect murine and human DIPG cell lines, produce oncolytic effect in a dose-dependent manner and express the corresponding functional ligand (+1BB, OX40L) on the cell membrane of infected cells (expressing them at 10 MOIs). As expected, viral replication was optimal in human cell lines but semipermissive in murine cells. In vivo, the intratumoral administration of armed oncolytic DIPG murine tumors, leading to long-term survivors. In addition, we analyzed the effect of the virus in the tumor microenvironment by flow cytometry and immunohistochemistry, indicating that there was a significant increase of immune infiltration in brains of treated mice. Moreover, the immune infiltrated showed a functional active phenotype. Although deeper characterization is needed, these data support that the infection of a positive immune modulator into Delta-24-RGD could improve the oncolytic effect of the virus by boosting the immune response, while maintaining a safe profile in immunocompetent models offering a feasible option treatment for DIPG.

**IMMU-07. “STROKE MIMICS” ARE NOT BENIGN IN IMMUNOCOMPROMISED CHILDREN**

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IMMU-09. MODULATING THE MYELOID POPULATION IN DIPG MYELOIDS WITH ONCOLYTIC VIRUS AND COMPLEMENT INHIBITORS SHOWS THERAPEUTIC EFFICACY

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Diffuse Midline Gliomas (DMGs), encompassing Diffuse IntrinsicPontine Gliomas (DIPGs), are aggressive pediatric brain tumors. Their current survival has not changed despite the combination of radiotherapy with targeted therapies emphasizing the urgent need for effective treatments. Recent research suggested that the DIPG tumor microenvironment is neither highly immunosuppressive nor inflammatory. Analyses showed the lack of infiltrating lymphocytes and the abundance of CD11b+ cells. TIM-3 (HAVCR2) is a member of the T-cell immunoglobulin and mucin domain family which is expressed on multiple immune cell types including T cells, NK cells, monocytes, dendritic cells and microglia, where it potently regulates not only adaptive immunity but also innate immunity. Therefore, the immune responses generated by this study is TIM-3 inhibitors could stimulate a cytotoxic immune effe
c
t and challenge several components in the tumor microenvironment including microglia, thereby providing a potential effective treatment for DMGs. In silico assessment of TIM-3 expression and genomic data showed a promising signature of this gene. Moreover, single-cell sequencing analyses of DIPG biopsies uncover its expression on tumor cells, especially in the OPCs compartment. In vivo efficacy studies showed that treatment with anti-TIM-3 antibody significantly increases the bearing survival in two DIPG immunocompetent orthotopic models (doubling the median), lead to long-term survivors (50%) and showed immune memory. Analyses of CD45+ populations in the tumor microenvim
ftment showed a significant increase in B, NK and CD8+ cells corresponding with a T-cell activate phenotype in treated mice. The potential therapeutic involvement of NK cells was certified using nude mice and functional studies. Involvement of microglia in currently being analysed. In summary, these data underscore TIM-3 as a potential target DIPGs and uncover the potential involvement of NKs and other immune mechanisms in the efficacy of anti-TIM-3 therapy.
Diffuse intrinsic pontine glioma (DIPG) is the leading cause of brain tumor-related death in children. It is characterized 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 through 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 inhibitors could constitute an actionable target, and second whether combining C5aR1 inhibitors with oncolytic virus could result in a superior antitumor immune response rather 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 vitro 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 survival of animals 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 with PMX205-24-ACT, which has an improved receptor cross-linking, leading 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 receptor (CD200AR). We have shown 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, 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 checkpoint connections are critical. 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 translatability 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 B7H3CAR 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 352 days from diagnosis following focal radiation and temozolomide, trimethylating agent and bevacizumab. Apheresis and manufacturing produced a second-generation B7H3CARs with a methotrexate-resistant human DHFR mutein (huDHFR®; L22E,F31S) in a single transcription in combination with the B7-H3-specific CAR and EGFR, each separated by a CD28 transmembrane and intracellular 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 lasting ~12–48 hours after each infusion. Following the 8 fusions of 1x10^6 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 ‘blunt,’ immune checkpoint inhibitors are unlikely to mediate therapeutic response. Alternately, immunogenic response can be induced both against pediatric gliomas with mRNA cancer vaccines. Messenger RNA represents a paradigm shift in vaccination (i.e. COVID-19) given its flexibility, commercialization, and propensity to confer rapid protection with only a single injection. 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 Krr158b brain tumors and activate dendritic cells that suppress 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 rat 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 are 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 (PNOCL20 trial, study NCT04573140).