(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 mice with medulloblastoma by crossing hGFAP-Cre and SmoM2 mouse lines. 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 to 37 days (n=10) (p=0.003508). 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 therapy, can produce 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 the optimal dose regimen, and 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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Diffuse Intrinsic Pontine Gliomas (DIPG) are aggressive pediatric brain tumors that arise in the pons of children, being the leading cause of pediatric death caused by cancer. We have previously demonstrated that Delta-24-RGD administration is safe and efficacious in DIPG preclinical models, indicating that it could be a good candidate as therapeutic approach for DIPG. However, our data underscore that there is still room to improve the anti-DIPG effect obtained with Delta-24-RGD. For that purpose, we have constructed three new virus by engineering Delta-24-RGD with different T cell activators: 4-1BBL (Delta-24-ACT), OX40-L (Delta-24-RGDOX) and GITRL (Delta-24-GREAT), to further increase the immune response generated by the viral effect. *In vitro*, 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 (4-1BBL, OX40L or GITRL) in the membrane of infected cells (almost 100% of 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 viruses was safe and significantly increased survival of mice bearing orthotopic 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, which indicated 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 show that the incorporation 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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Objective: To determine the clinical variances between strokes and stroke mimics in a pediatric immunocompromised population that consists of children with central nervous system (CNS) and non-CNS malignancies and a history of solid organ transplantation. 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 if the diagnosis necessitated changes in 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 neurotoxicity (29%), tumor progression (19%), and seizures/posterior reversible encephalopathy syndrome (13%). Patients meeting inclusion criteria with strokes and stroke mimics presented similarly, with the exception of altered mental status, which was more prevalent in patients with strokes than stroke mimics ($p = 0.03$). One child received hyperacute thrombectomy for stroke. Only 58% of children with stroke mimics had complete resolution of their presenting neurologic symptoms. Children with strokes and stroke mimics had similar mortality incidences of 33% and 37%, respectively. Conclusions: Although all acute neurologic changes in immunocompromised children are not strokes, stroke mimics in this population are neither benign nor self-limited and carry long-term neurologic morbidity and mortality. This study highlights the utility of an acute stroke evaluation infrastructure and the need for acute and long-term neurology involvement in the care of these patients.

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

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Diffuse Midline Gliomas (DMGs), encompassing Diffuse Intrinsic Pontine Gliomas (DIPGs), are the most aggressive pediatric brain tumors. Their meagre 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. These 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 protein family which is expressed on multiple immune cell types including T cells, T_{regs}, NK cells, monocytes, dendritic cells and microglia, where it potently regulates not only adaptive immunity but also innate immunity. Therefore, the central hypothesis of this study is that TIM-3 inhibitors could stimulate a cytotoxic immune effect 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 in a DIPG datasets showed a robust expression 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 increase the overall survival in two DIPG immunocompetent orthotopic animal models (doubling the median), lead to long-term survivors (50%) and showed immune memory. Analyses of $CD45^+$ populations in the tumor microenvironment 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.

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

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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).