IMMU-14. COMPUTATIONAL DECONVOLUTION OF TUMOR-INFILTRATING IMMUNE COMPONENTS IN PEDIATRIC NERVOUS SYSTEM TUMORS

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Introduction: In the last decade, checkpoint inhibitor-based immunotherapy has been a groundbreaking development in the treatment of cancer. However, only a subset of patients treated with immune checkpoint inhibitors show long-lasting clinical benefit. Studies showed the tumor immune microenvironment (TME) as a particularly important factor influencing treatment response, critical for the design of other or combinatorial immunotherapy treatment strategies. Extensive research has been performed in the adult cancer field to unravel its immunogenomic aspects. However, in pediatric cancer this insight into tumor-infiltrating immune components is still lacking. This study aims to provide insight into the landscape of the immune microenvironment in pediatric primary nervous system tumors. Methods: Bulk RNA-seq data of 936 pediatric primary solid tumors acquired from multiple international initiatives including Therapeutically Applicable Research To Generate Effective Treatments (TARGET), the International Cancer Genome Consortium (ICGC) and the Children's Brain Tumor Tissue Consortium (CBTTC) were included in this study. We applied computational tumor immune microenvironment deconvolution, repurposed RNA-seq data to recover infiltrating T- and B-cell clonotypes and studied checkpoint gene expression across pediatric neural tumors. Results: Among pediatric neural tumors, embryonal tumors with multilayered rosettes (ETMR) and medulloblastomas (MB) were least immune infiltrated. Neuroblastomas (NBL) had the highest T-cell infiltration among pediatric cancers, while atypical teratoid/rhabdoid tumors (ATRT) had the highest levels of CD8 T cell infiltration among pediatric CNS tumors. While tumor mutational burden (TMB) was associated with immune cell infiltration in adult lung cancers and melanomas, we found no significant associations in pediatric cancers. The majority of NBL samples expressed *LAG3*, but ~10% of samples had elevated levels of *TIM3* gene expression, suggesting a distinct mode of immunosuppression in this subset.

IMMU-15. QUANTIFYING INTRATHECAL DRUG DELIVERY UTILIZING PROGRAMMABLE VENTRICULOPERITONEAL SHUNTS

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Background: Programmable ventriculoperitoneal (pVP) shunts are increasingly utilized for intraventricular chemotherapy, radioimmunotherapy, and/or cellular therapy. Shunt adjustments allow optimization of thecal space drug concentrations with minimization in the peritoneum. Drug delivery quantification using several types of pVP shunts has not been reported. Methods: We performed a retrospective analysis on patients with CNS tumors and pVP shunts at Memorial Sloan Kettering Cancer Center from 2003–2020, noting shunt model. CSF flow through the pVP shunt was evaluated using In-111-DTPA scintigraphy at approximately 4 hours and 24 hours after injection. pVP shunts were calibrated pre-injection to minimize peritoneal flow and re-calibrated to baseline setting 4–5 hours following injection. Scintigraphy studies quantified ventricular-thecal and peritoneal drug activity at these 2 time points. Results: Twenty-one CSF flow studies were administered to 15 patients, ages 1–27 years. Diagnoses included medulloblastoma (N=10), metastatic neuroblastoma (N=3), pineoblastoma (N=1), and choroid plexus carcinoma (N=1). pVP shunt models included Aesculap Miethke proGAV (N=3), Aesculap Miethke proGAV2.0 (N=3), Codman HAKIM (N=2), Codman Certas Plus (N=1), Medtronic STRATA (N= 5), and Sophysa Polaris (N= 1). All 21 studies (100%) demonstrated ventriculothecal drug activity. 29% (6 of 21) of the studies had no peritoneal uptake visible by imaging. 73% (16 of 21) of the studies had minimal peritoneal uptake (<12%), and 24% (5 of 21) demonstrated moderate peritoneal uptake (12–37%). pVP shunt models measuring minimal to no peritoneal uptake included: Aesculap Miethke proGAV (N=2), Aesculap Miethke proGAV2.0 (N=3), Codman HAKIM (N=2), Codman Certas Plus (N=1), Medtronic STRATA (N= 3), and Sophysa Polaris (N= 1). Conclusions: Successful drug delivery to the ventriculo-thecal space can be accomplished using pVP shunts: 80% of studies have minimal (<12%) peritoneal drug activity. Though efficacy varies by shunt model, low numbers preclude conclusions regarding model superiority. CSF flow scintigraphy studies reliably assess drug distribution.

IMMU-16. TARGETING GLYPICAN 2 (GPC2) ON PEDIATRIC MALIGNANT BRAIN TUMORS WITH MRNA CAR T CELLS

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Glypican 2 (GPC2) is a cell-surface oncoprotein initially identified in neuroblastoma, retinoblastoma, and medulloblastoma as an ideal target for immunotherapy (Cancer Cell, 2017). Here we evaluated *GPC2* expression across the spectrum of pediatric brain tumors using RNA sequencing from specimens in the Children's Brain Tumor Network (CBTN). High *GPC2* expression, defined as >10 FPKM, was found in 100% of embryonal tumors with multilayered rosettes (ETMRs) (n=6), 95% of medulloblastomas (n=122), 86% of other embryonal tumors (n=21), 50% of choroid plexus carcinomas (n=4), 42% of high grade gliomas (HGG) (n=117), and 37% of diffuse midline gliomas (DMG) (n=65). Within medulloblastoma subtypes, group 4 tumors had the highest expression, and within the HGG tumor cohort H3.3 G34 mutated gliomas had the highest *GPC2* expression. High GPC2 protein expression was validated with medulloblastoma and HGG/DMG primary tumors and cell lines using IHC, Western blot, and flow cytometry. We next developed two potent CAR T cell constructs using the D3 specific scFv directed against GPC2 for testing in brain tumor models. GPC2-directed CAR T cells were tested *in vitro* against medulloblastoma and HGG cells lines, and *in vivo* using two patient-derived medulloblastoma xenograft models: Rcmb28 (group 3) and 7316-4509 (group 4). GPC2-directed mRNA CAR T cells induced significant GPC2-specific cell death in medulloblastoma and HGG cellular models with concomitant T cell degranulation compared to CD19-directed mRNA CAR T cells. *In vivo,* GPC2-directed mRNA CAR T cells delivered locoregionally induced significant tumor regression measured by bioluminescence after 4–6 intratumoral infusions of 4 x 10^6 CAR T cells ($p<0.0001$ for Rcmb28, $p<0.05$ for 7316-4509). No GPC2-directed CAR T cell related toxicity was observed. GPC2 is a highly differentially expressed cell surface protein on multiple malignant pediatric brain tumors that can be targeted safely with local delivery of mRNA CAR T cells.

IMMU-17. USE OF MRNA FOR SAFE AND EFFECTIVE GD2-DIRECTED CAR T CELLS TO TREAT DIFFUSE MIDLINE GLIOMAS

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Chimeric antigen receptor (CAR) T cells targeting the disialoganglioside GD2 have garnered interest as an effective therapeutic for treating diffuse midline glioma (DMG). However, prior studies raised significant concerns of neurotoxicity and fatality when using virally transduced CAR T cells against these midline tumors. Building upon our prior work optimizing mRNA for use in CAR T cells (Hum Gen Ther, 2019), we hypothesized transient GD2-directed mRNA CAR T cells could be successfully employed for safe and effective treatment of DMG. GD2-directed CAR T cells were created using mRNA encoding the 14G2a single chain variable fragment paired with 41BB and CD3-zeta co-stimulatory domains and transfected into human T cells. GD2-directed CAR T cells were tested against a panel of DMG cell lines and two murine xenograft models of DMG: 7316-6349 and SU-DIPG13P*. In all DMG cellular models, GD2-directed mRNA CAR T cells induced significant tumor cell death compared to CD19-directed mRNA CAR T cell controls. *In vivo,* mRNA CAR T cells were delivered locoregionally using an indwelling infusion catheter to allow for repeated dosing. Four intratumoral doses of 5 x 10^6 GD2-directed mRNA CAR T cells induced significant tumor regression measured by bioluminescence in DMG model 7316-6349 ($p<0.0001$). In addition, GD2-directed mRNA CAR T cells prolonged survival of mice harboring the aggressive DMG model SU-DIPG13P* by 61% (mean survival 29 days versus 18 days, $p<0.01$) following four intratumoral doses of 4 x 10^6 CAR T cells. No GD2-directed CAR T cell treatment-related deaths or toxicities were observed. These data highlight the utility of

using mRNA to titrate CAR T cell therapy in the brain, and establish GD2-directed mRNA CAR T cells as a safe and effective method for treating DMG.

LOW GRADE GLIOMAS

LGG-01. *NF1* MUTATION DRIVES NEURONAL ACTIVITY-DEPENDENT OPTIC GLIOMA INITIATION

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Neurons have recently emerged as essential cellular constituents of the tumor microenvironment, where their activity increases the growth of a diverse number of solid tumors. While the role of neurons in tumor progression has been previously demonstrated, the importance of neuronal activity to tumor initiation is less clear, particularly in the setting of cancer predisposition syndromes. In the Neurofibromatosis type 1 (NF1) cancer predisposition syndrome, in which tumors arise in close association with nerves, 15% of individuals develop low-grade neoplasms of the optic pathway (optic gliomas) during early childhood, raising the intriguing possibility that postnatal light-induced optic nerve activity drives tumor initiation. Here, we employ an authenticated murine model of *Nf1* optic glioma to demonstrate that stimulation of optic nerve activity increases optic glioma growth, while decreasing optic nerve activity via light deprivation prevents tumor formation and maintenance. By manipulating environmental light to modulate optic pathway (retinal) neuron activity, we show that *Nf1* optic glioma initiation depends on neuronal activity during a developmental period susceptible to tumorigenesis. Germline *Nf1* mutation in retinal neurons results in aberrantly high optic nerve neuroligin-3 (Nlgn3) shedding in response to retinal neuronal activity. Moreover, genetic *Nlgn3* loss or pharmacologic inhibition of Nlgn3 shedding blocks murine *Nf1* optic gliomagenesis and progression. Collectively, these studies establish an obligate role for neuronal activity in the development of certain brain tumors, elucidate a therapeutic strategy to reduce optic glioma incidence or mitigate tumor progression, and underscore the role of *Nf1* mutation-mediated dysregulation of neuronal signaling pathways in the NF1 cancer predisposition syndrome.

LGG-02. PEDIATRIC LOW-GRADE GLIOMA RISK STRATIFICATION IN THE MOLECULAR ERA

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Background: Pediatric low-grade gliomas (LGG), in particular those not amenable to surgical resection, are a therapeutic challenge owing to their heterogeneity in clinical behavior. Identification of the RAS/MAPK pathway as a universal feature of these tumors has led to an improved understanding and the development of targeted therapeutics. We examined the impact of known biological and novel molecular risk factors on patient outcomes at our institution. Methods: We retrospectively reviewed risk factors and clinical outcomes in 38 LGG cases diagnosed by histopathology at Norton Children's Hospital in Louisville, KY, USA from March 2015 to Jan 2019. Progression free survival (PFS) rates were generated using the Kaplan-Meier method. Log-rank tests and hazard ratios were used to identify prognostic factors by univariable analysis. Results: Among previously described biological risk factors, subtotal resection/biopsy only (HR 3.67, p=0.0257), non-WHO Grade I histology (HR 3.34, p=0.0101), and infant age (< 3 years) (HR 4.19, p=0.0031) were associated with shorter PFS. Brainstem location had no significant impact on PFS. (HR 0.86, p=0.8071). H3K27M mutant status was predictably associated with worse PFS (HR 5.86, p=0.0012). BRAF v600e mutant status, however, was not associated with inferior outcomes. On the contrary, in our study population, BRAF v600e mutant status had a suggested protective effect (HR 0.14, p=0.0247). Patients with 2 or more oncogenic driver mutations demonstrated worsened PFS (HR 4.78, p=0.0059). We utilized the following scoring system for risk stratification: 1 point was allocated for each of the above biological and molecular risk factors except for H3K27M, which was allocated 3 points. A score of < 3 was designated low risk. Non-low risk classification was associated with significantly inferior PFS (median PFS 13 vs. 62 mos, HR 4.26, p=0.0012). Conclusion: We herein demonstrate the utility of a combined biological and molecular risk classification for pediatric LGG.

LGG-03. LONG-TERM FOLLOW UP OF TARGETED THERAPY IN PEDIATRIC LOW-GRADE GLIOMAS: THE DANA-FARBER/BOSTON CHILDREN'S EXPERIENCE

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Background: Pediatric low grade gliomas (pLGGs) are the most common central nervous system (CNS) tumor in children and characterized by alterations in the MAPK pathway. Standard of care is not well defined, and treatment has evolved over the last decade to include molecular targeted therapies. The impact of targeted agents on the natural history of pLGGs remains unknown. We present a retrospective review of patients receiving targeted agents integrated with molecular profiling. Methods: We performed an IRB-approved, retrospective chart review of pLGGs treated with off-label use of dabrafenib, vemurafenib, everolimus, and trametinib at Dana-Farber/Boston Children's Cancer and Blood Disorders Center from 2010 to 2020. Results: Forty-nine patients were identified (dabrafenib n=9, everolimus n=27, trametinib n=10, and vemurafenib n=3). All patients receiving BRAF inhibitors harbored BRAF V600E mutation. Targeted agent was used as first-line therapy for 25% of patients, while for 31% of patients, targeted agent was second-line therapy. The median time from diagnosis to targeted therapy initiation was 4.76 years (0.10 – 23.77), median duration of targeted therapy was 0.79 years (0.01 – 4.87), median time to subsequent therapy post first-line targeted therapy was 0.2 years (0.01 – 3.33), and overall median follow-up for the entire cohort was 3.09 years (0.36 – 11.87). The 1-year, 3-year, and 5-year EFS from targeted therapy initiation was 58.0%, 32.2%, and 26.9%, respectively. Survival analyses by molecular subgroup and agent were performed. Reasons for cessation of targeted therapy included toxicities, progression, and/or planned end of therapy. Conclusions: Further efforts are ongoing to perform volumetric analysis of growth rates before, during, and after treatment. While targeted molecular therapies show great promise, it will be critical to understand how these agents alter the natural history of pLGGs, particularly in the context of genomic profiling.

LGG-04. MULTIOMIC ANALYSIS OF MAPK PATHWAY ACTIVITY IN PEDIATRIC PILOCYTIC ASTROCYTOMA

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Introduction: Pilocytic astrocytomas (PA) are the most common pediatric brain tumors. They are characterized by MAPK pathway alterations, leading to its constitutive activation and modulating the balance between cell proliferation and the oncogene-induced senescence (OIS) sustained by senescence-associated secretory phenotype (SASP) factors. This makes PA suitable for MAPK inhibitor (MAPKi) therapies, showing encouraging results in phase 1/2 clinical trials. Little is known about the molecular implications of MAPK downregulation in the proliferating and senescent compartments. Methods: DKFZ-BT66 PA cells derived from a primary KIAA:BRAF-fusion positive PA cell line, were used as model system. Gene expression and phospho-proteomic datasets were generated from DKFZ-BT66 cells, in both the proliferative and senescent states, and treated with the MEKi trametinib for different time-spans. A time course analysis based on differentially expressed genes was performed, followed by a single-sample gene set enrichment analysis (ssGSEA). Analysis of the phospho-proteomic data is ongoing. Results: Differential gene expression analysis revealed that MEK inhibition leads to the inhibition of the OIS-SASP gene program in senescent DKFZ-BT66. ssGSEA showed that most