IMMU-19. HDAC INHIBITORS SENSITIZE MYC-AMPLIFIED MEDULLOBLASTOMA TO IMMUNOTHERAPY BY ACTIVATING THE NF-KB PATHWAYS

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Medulloblastoma is the most common malignant brain tumor in childhood and comprises four distinct molecular subgroups with further layers of intertumoral heterogeneity. Amplification of the oncogene MYC drives tumorigenesis and constitutes a hallmark feature underlying Group 3 biology. Employing our in-house drug screening pipeline, we evaluated a library of epigenetic inhibitors (n=78) in various brain tumor cell lines followed by a secondary HDACi library (n=20) screen, we identified the clinically established, class I selective HDACi CI-994 as the compound with the most preferential antitumoral effect in MYC-driven medulloblastoma. We confirmed that the inhibitor response was in part MYC-dependent as our lentiviral-based MYC-overexpression model showed higher sensitivity towards CI-994 treatment as compared to the isogenic control with low endogenous MYC expression. CI-994 showed significant antitumoral effects at the primary site and at the metastatic compartment in two orthotopic mouse models of MYC-driven medulloblastoma. RNA sequencing profiling of tumor cells treated with CI-994 at IC50 revealed an up-regulation of multiple innate inflammatory pathways like NFκB, TLR4, Interferon-gamma, and TGFbeta. Flow cytometry analysis revealed an increased surface expression of MHC-I. We combined CI-994 with an anti-body against the innate checkpoint CD47 which acts as a "don't eat me" signal previously shown by us to have significant anti-tumor activity against MYC-driven MB. Combining CI-994 with anti-CD47 shows a significant increase in macrophage-mediated phagocytosis of tumor cells and a significant increase in the survival of tumor-bearing mice.

IMMU-20. EVALUATION OF CAR T CELLS IN EPENDYMOMA

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BACKGROUND: Ependymoma is the third most common pediatric brain tumor and current treatment still results in a 10-year relapse rate of over 70% in the highest risk groups. The treatment refractory nature of ependymoma to standard therapies strongly supports the development of novel interventions. Ependymoma tumor cells express HER2 and there are active clinical trials treating children with ependymoma using local delivery of second-generation HER2 CAR T cells. METHODS: Two high-risk patient-derived ependymoma cell lines, MAF811 and MAF928, that display HER2 surface expression are used for testing. We tested second-generation HER2-BBz CAR T cells in vitro and in vivo. RESULTS: HER2 CAR T cells effectively kill ependymoma tumor cells in culture, but this strategy cannot eradicate the same tumor cells in mice when implanted in the fourth ventricle of the brain. HER2 CAR T cells proliferate and traffic into the tumor, but this causes a dramatic influx of immune cells, tumor swelling and lethal toxicity in a subset of mice. Mice that survive this initial tumor swelling, display significant tumor shrinkage but all tumors eventually start growing again. Ependymoma tumor cells release high amounts of inflammatory chemokines that strongly attract neutrophils and monocytes to the tumor, compared to other brain tumors, and can downregulate HER2 expression to escape recognition by CAR T cells. CONCLUSION: The immunosuppressive microenvironment as well as tumor heterogeneity make HER2 CAR T cells ineffective in ependymoma. Studying these two hurdles in CAR T cell therapy is critical to effectively treat brain tumors with CAR T cells.

IMMU-21. INVESTIGATION OF WHITE BLOOD CELL CHARACTERISTICS IN CSF SAMPLES AT PEDIATRIC BRAIN TUMOR DIAGNOSIS

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BACKGROUND: There has been a recent surge in investigation of immunity and immunotherapy, but their role in pediatric brain tumors is incompletely defined. We hypothesized that investigating an understudied dataset, WBC and differential results in CSF drawn at the time of pediatric brain tumor diagnosis to look for microscopic metastases, would provide insight into the role of immunology and potential for immunotherapy in these diseases and correlate with prognosis and/or metastasis. METHODS: We conducted a retrospective comparison analysis of CSF values in 349 patients at our institution from samples drawn within 60 days of initial CNS tumor diagnosis from 1998–2018. We examined total nucleated cell count, absolute counts and percentages for WBC subtypes. We compared CSF values by tumor cell presence, patient vital status, and disease group: atypical teratoid rhabdoid tumor, ependymoma, germinoma, high-grade glioma (HGG), low-grade glioma (LGG), medulloblastoma, non-germinomatous germ cell tumor, and other embryonal tumors (OET). We used Wilcoxon and Kruskal-Wallis tests for comparisons. RESULTS: Overall, higher lymphocyte percentage (p=0.002) and lower monocyte percentage (p=0.007) were associated with survival. WBC characteristics did not differ significantly based on tumor cell presence. Compared to medulloblastoma, ependymoma showed a more active CSF immune response, while LGG, HGG, and OET showed a less active response, based on total WBC and/or absolute neutrophil count (p=0.001–0.007). CONCLUSIONS: Higher lymphocyte and lower monocyte percentages in CSF correlated with better prognosis overall; causality requires further investigation. Tumor subtypes varied in their immune stimulation, offering potential insight into which will be amenable to immunotherapy.

IMMU-22. PHASE IB IMMUNOTHERAPY CLINICAL TRIAL WITH THE USE OF AUTOLOGOUS DENDRITIC CELLS PULSED WITH AN ALLOGENIC TUMORAL CELL LINES LYSATE IN PATIENTS WITH NEWLY DIAGNOSED DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG)

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BACKGROUND: Diffuse intrinsic pontine glioma (DIPG) is a lethal condition, and therefore novel approaches are needed. Monocyte-derived dendritic cells (mDCs) pulsed with tumor antigens, as professional antigen-presenting cells, are a promising strategy for immunotherapy of invasive brain tumors. METHODS: Our Ib pilot study explored the use of immunotherapy with mDCs for the treatment of newly diagnosed DIPG. Patient´s mDCs were extracted after irradiation and were primed with an allogenic tumor lysate from five patients with K27M-mutated DIPGs. The principal goal of this study was to establish the feasibility and safety of the intradermic administration of these mDC vaccines in patients with DIPG. In the absence of progression, patients received maintenance boosts of tumor lysate. Additionally, we evaluated the non-specific and antitumoral immune response generated in peripheral blood mononuclear cells (PBMC) and in cerebrospinal fluid (CSF) cells. RESULTS: Nine patients were included in the study (2016–2018). Vaccines fabrication was feasible and administered in all cases without grade 3 or 4 toxicities. KLH (9/9 patients) and antitumor (8/9 patients) specific responses were identified in PBMC. Immunological responses were also confirmed in T-lymphocytes from the CSF of two patients. Twenty-four month overall survival and progression free survival was 33.3% (95 % CI 13.2% to 84.0 %) and zero, respectively. DISCUSSION: These results demonstrate that mDC vaccination is feasible, safe, and generates a DIPG-specific immune response detected in PBMC and CSF. There was a trend in improved OS when compared to historic controls. This strategy shows a promising immunotherapy backbone for future combination schemas.

IMMU-23. A NOVEL MASS CYTOMETRY-BASED MULTI-PARAMETER CHARACTERIZATION OF NEOANTIGEN-REACTIVE CD8+ T-CELLS IN PATIENTS PARTICIPATING IN PNOC007 H3.3K27M PEPTIDE VACCINE CLINICAL TRIAL

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BACKGROUND: We have identified an HLA-A*02:01-restricted neoantigen epitope encompassing the H3.3K27M mutation and implemented a multi-center clinical trial of the peptide vaccine through the Pacific Pediatric Neuro-Oncology Consortium (PNOC007) for patients with diffuse midline glioma (DMG), including diffuse intrinsic pontine glioma (DIPG). We sought to characterize vaccine-reactive CD8+T-cells subpopulations using their precise activation and developmental status to find their associations with clinical outcomes. METHODS: Mass cytometry (CyTOF) analysis was performed on patient-derived peripheral blood mononuclear cells collected at baseline as well as pre-specified time points throughout the study. Each cell subtype was characterized via tSNE-clustering based on their expression profiles and quantified as a fraction of total CD45+cells. H3.3K27M-reactive CD8+T-cells were evaluated using an H3.3K27M-

HLA-A2 dextramer along with a panel of T-cell and myeloid markers. RESULTS: Among all 29 patients enrolled, we analyzed samples from all 19 DIPG and 9 of 10 non-brainstem DMG cases, of which 18 had longitudinal samples available (range: 2–5). Utilizing a novel CyTOF-based immunomonitoring platform, the expansion of H3.3K27M-reactive CD8+T-cells, defined as a 25% increase at any time-point relative to baseline, was observed in 7 of these 18 patients. Survival analyses indicated that the expansion of H3.3K27M-reactive CD8+T-cells, particularly the effector-memory phenotype, positively correlated with longer overall survival (OS) (median: 16.1 vs 9.7 months, p=0.03), whereas an abundance of early and monocytic myeloid-derived suppressor cells at baseline correlated with shorter OS among DIPG patients (9.5 vs 14.3 months, p=0.002). CONCLUSION: Our novel immuno-monitoring approach offers insight into how vaccine-induced immune responses impact clinical outcomes.

IMMU-26. DISEASE CONTROL IN A PEDIATRIC PATIENT WITH NEWLY DIAGNOSED GLIOBLASTOMA MULTIFORME (GBM) AND SOMATIC HIGH MICROSATELLITE INSTABILITY (MSI-H) WITH PD-1 INHIBITOR NIVOLUMAB (NIVO) ONLY AND NO FOCAL RADIOTHERAPY (RT)

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Immune checkpoint inhibitors that target programmed death receptor-1 (PD-1) have recently been shown to be a promising option for the management of recurrent mismatch repair (MMR) deficient GBM following radiotherapy. We report a case of a 9-year-old boy who presented with a 6 week history of frontal headaches and was found to have a left frontal lobe mass. Pathology obtained from a gross total resection (GTR) was consistent with classic GBM, WHO Grade IV. Neuroimaging four weeks following initial resection was remarkable for local recurrence. The patient underwent another GTR of the tumor at our center. While pathology again confirmed GBM, GlioSequencing of tumor tissue from second resection showed MSI-H, *NF2* mutation p.R338H, *NF1* mutations p.R2450* and pI193Yfs*11, *TP53* mutations p.R213* and p.R273C, *EGFR* mutation, and multiple variants of uncertain significance. Germline testing was negative for MMR deficiency or other deleterious mutations. Parents opted to defer radiotherapy and consented to monotherapy treatment with Nivolumab (Opdivo, BMS pharmaceuticals, USA), a PD-1 inhibitor, at a dose of 3 mg/kg administered every two weeks. Our patient is now 22 months post-second resection and continues to receive Nivolumab without evidence of recurrent disease or adverse autoimmune effects from PD-1 blockade. He has remained in school with good academic performance and has exhibited no regression of functional status during the entirety of his treatment course. This case provides evidence of possible efficacy of PD-1 blockade without focal radiotherapy in this child with GBM and somatic MSI instability.

IMMU-27. ANALYSIS OF IMMUNE SIGNATURES IN PEDIATRIC GLIOBLASTOMAS FOR PATIENT STRATIFICATION TO IMMUNOTHERAPY

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BACKGROUND: Pediatric glioblastoma (pGBM), despite being relatively rare (incidence rate: 0.5/100,000), are a leading cause of cancer deaths in children with a median overall survival of 9–15 months. In recent years, immunotherapy has emerged as one of the more promising advances in oncology, with impressive response rates reported in several malignancies. Effective application of immunotherapy in brain tumors depends upon a better understanding of the immune cell phenotype and mechanisms of immunosuppression in these tumors. This understanding will allow for the selection of patient population who are most likely to benefit from immunotherapeutic approaches. MATERIAL AND METHODS: In order to determine the frequency, distribution, and phenotype of tumor-infiltrating immune cells in pGBMs, we undertook an immunohistochemical survey on 19 recurrent pGBMs for CD3, CD8, CD4, CD163, PD-1, PD-L1, and FoxP3; RNA-Seq was also performed on a subset of 9 cases. Distribution of lymphocytes (LYMPHS) was recorded as intratumoral (IT) or perivascular (PV). RESULTS: The analysis indicates intratumoral CD3+ LYMPHS are commonly <5% of tumor cell mass; however, approximately half (10/19) of these recurrent pGBM have infiltrates that range from 5 to 30% CD3+ LYMPHS. Of these, 4/10 CD3+ tumors exhibit brisk CD8+ infiltrates that are associated with PD-L1+ tumor cells. These tumors with brisk CD3+/CD8+ LYMPHS and PD-L1+ tumor cells were associated with longer survivals. The data were confirmed by RNA-seq analysis. CONCLUSION: PD-L1+ pGBMs associated with CD3+/CD8+ LYMPH infiltrates deserve further investigation as candidates for immunotherapy.

IMMU-28. IMMUNOGENOMIC ANALYSIS REVEALS LGALS1 CONTRIBUTES TO THE IMMUNE HETEROGENEITY AND IMMUNOSUPPRESSION IN GLIOMA

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Mutualistic and dynamic communication between tumour cells and the surrounding microenvironment accelerates the initiation, progression, chemoresistance and immune evasion of glioblastoma (GBM). However, the immunosuppressive mechanisms of GBM has not been thoroughly elucidated to date. We enrolled six microenvironmental signatures to identify glioma microenvironmental genes. The functional enrichment analysis such as ssGSEA, ESTIMATE algorithm, Gene Ontology, Pathway analysis is conducted to discover the potential function of microenvironmental genes. In vivo and in vitro experiments are used to verify the immunologic function of LGALS1 in GBM. We screen eight glioma microenvironmental genes from glioma databases, and discover a key immunosuppressive gene (LGALS1 encoding Galectin-1) exhibiting obviously prognostic significance among glioma microenvironmental genes. Gliomas with different LGALS1 expression have specific genomic variation spectrums. Immunosuppression is a predominate characteristic in GBMs with high expression of LGALS1. Knockdown of LGALS1 remodels the GBM immunosuppressive microenvironment by down regulating M2 macrophages and myeloid-derived suppressor cells (MDSCs), and inhibiting immunosuppressive cytokines. Our results thus implied an important role of microenvironmental regulation in glioma malignancy and provided evidences of LGALS1 contributing to immunosuppressive environment in glioma and that targeting LGALS1 could remodel immunosuppressive microenvironment of glioma.

IMMU-29. AIF1 IS A PROGNOSTIC BIOMARKER AND CORRELATED WITH IMMUNE INFILTRATES IN GLIOMAS

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Gliomas remain highly variable clinical behaviors, leading to emerging studies to identify prognostic factors. AIF1 (Allograft Inflammatory Factor 1) is critical for promoting both macrophage- and dendritic cells (DCs)-mediated inflammatory response and growth of vascular smooth muscle cells and T-lymphocytes. Through comparative analyses of primary LGG patients from The Cancer Genome Atlas (TCGA) dataset and Chinese Glioma Genome Atlas(CGGA) dataset, we reported that the expression level and methylation level of AIF1 gene vary among glioma patients and AIF1 expression or gene body methylation is significantly associated with glioma patient survival. Cox regression results confirmed that AIF1 played an independent predictor of survival in lower-grade glioma(LGG), with a cox coefficient of 0.251 indicating a worse prognosis. Moreover, AIF1 expression was positively correlated with infiltrating levels of CD4+ T and B cells, macrophages, neutrophils, and DCs in LGG and glioblastoma(GBM). AIF1 expression also showed strong correlations with specific immune cell markers in LGG and GBM. In addition, AIF1 expression potentially contributed to the regulation of glioma-associated macrophages and microglia. In conclusion, our findings suggested that AIF1 was correlated with prognosis and immune infiltrating levels, and it can be used as a prognostic factor in gliomas.

IMMU-30. UPREGULATED T CELL AND INTERFERON-Γ-RELATED GENE EXPRESSION IS ASSOCIATED WITH INCREASED SURVIVAL IN RECURRENT PEDIATRIC HIGH-GRADE GLIOMA

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