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-