largely unknown. We evaluated therapeutic effects of anti-PD-1, temozolomide (TMZ), and their combination in an orthotopic murine GBM model. The phenotype, number, and composition of lymphocytes were evaluated using flow cytometry. Transcriptional profiles of tumor tissues were analyzed using microarrays. Generation of antitumor immunological memory was investigated upon rechallenge. Combined treatment with anti-PD-1 and TMZ yielded synergistic antitumor efficacy in the presence of donor-originated PD-1^{+}CD8^{+} T cells *in vitro*, necessitating *in vivo* validation. Whereas TMZ did not rescue GBM-implanted mice, anti-PD-1 completely eradicated GBM in 44.4% of mice, and combination of anti-PD-1 and TMZ in all mice. Anti-PD-1 significantly increased the number of tumor-infiltrating lymhpocytes (TILs), and reduced frequencies of exhausted T cells and regulatory T cells. However, combining TMZ with anti-PD-1 significantly decreased the number of TILs, which was also observed with TMZ treatment alone. A transcriptome analysis of tumor tissues revealed that anti-PD-1 monotherapy perturbed immune-related genes, distinctly with combined therapy. Upon rechallenge, tumor growth was not observed in mice cured by anti-PD-1 monotherapy, whereas tumors regrew in the combination group. Furthermore, an analysis of peripheral blood revealed that antitumor memory T cells were generated in mice cured by anti-PD-1 monotherapy, not in the combination group. PD-1 blockade induces long-term therapeutic response, and combination with TMZ further enhances antitumor efficacy. However, immunological memory is provoked by anti-PD-1 monotherapy, not by combined therapy.

IMMU-14. THERAPEUTIC MODULATION OF THE PHAGOCYTIC AXIS SPARKS ANTI-TUMOR CD8 T CELL RESPONSES IN GLIOBLASTOMA

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As the most common primary brain tumor in adults, Glioblastoma (GBM) remains a major unmet medical need. With current treatment strategies, the median survival remains approximately 15 months, and recurrence occurs in nearly all cases. In this study we examine a novel role for the DNA-methylating agent temozolomide (TMZ) as an activator of innate immunogenicity in GBM. TMZ-mediated DNA damage promotes calreticulin (CRT) translocation to the plasma membrane of cancer cells where it functions as a driver of phagocytosis. Ancillary blockade of anti-phagocytic signaling through Cluster of Differentiation 47 (CD47) further enhances tumor cell uptake by bone-marrow derived macrophages (BMDM). Together these agents promote maturation of BMDM into antigen presenting cells (APCs), capable of initiating effector T cell responses in vitro. We recapitulate these findings in immune-competent preclinical models of GBM, where combination therapy significantly prolongs survival in a cytotoxic CD8+ T cell dependent manner. The results of this study indicate that phagocytic axis modulation is a novel strategy to reprogram the innate immune microenvironment, shifting the dynamic towards an 'inflamed' tumor phenotype. This novel approach to immunotherapy in GBM is highly translational and warrants further investigation in the clinical setting.

IMMU-15. ENGINEERED-DRUG RESISTANT GAMMA-DELTA (γδ) T CELLS COMBINED WITH IMMUNE CHECKPOINT BLOCKADE AUGMENTED KILLING OF CANCER CELLS

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We have previously shown the potential for dramatic improvement in overall survival in immunodeficient mice bearing patient-derived xenograft (PDX) models of primary and temozolomide (TMZ)-resistant GBM when treated using a combination of intracranial therapy with *ex-vivo* expanded/activated MGMT-engineered γδT cells and simultaneous systemic TMZ. We have termed this approach Drug-Resistant Immunotherapy (DRI). TMZ upregulates γδT cell stress-antigen targets (NKG2DL) on primary and TMZ-resistant tumors making them more visible to effector γδT cells. In the present study, we sought to determine whether checkpoint inhibition would potentiate the effect of DRI in our PDX model system for glioma. Expanded/activated δT cells were evaluated for lysis of target cells K562 and disaggregated PDXT cells in the presence or absence of checkpoint inhibitors of PD-1, CTLA-4 and PD-L1 using a flow cytometric based killing assay. Cytotoxicity was increased by anti-CTLA-4 and anti-PD-L1 against JX22T PDXT, although a much more noticeable effect of blockade with anti-PD-1, anti-CTLA-4 and anti-PD-L1 was seen against K562. Anti-PD-1 combined with anti-CTLA-4 also showed a synergistic effect on JX22T and K562. Concurrently, we also demonstrated that although TMZ did not influence the already low expression of PD-L1 in disaggregated PDXT lines exposed to 200μM TMZ, immunohistochemical analysis of tumors from mice injected with 60mg/kg TMZ and examined 4h later showed upregulation of NKG2DL accompanied by modest upregulation of PD-L1. At the time of this writing, mice bearing JX12P and JX12T were still under examination. These early studies show that checkpoint inhibition can potentiate the cytotoxic activity of expanded/activated γδT cells. Also, TMZ may increase both the expression of NKG2DL and PD-L1 in some tumors. Taken together, our findings justify further exploration of combination DRI and checkpoint inhibition either by systemic administration, as part of the therapeutic graft, or both.

IMMU-16. GUADECITABINE (SGI-110) ENHANCES MHC class I AND TUMOR ANTIGEN EXPRESSION ON MURINE C57BL/6-SYNGENEIC GLIOMA AND DIPG MODELS

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Diffuse intrinsic pontine glioma (DIPG) is one of the most lethal pediatric brain tumors, with a median survival time of less than one year. As DIPG tumors are insensitive to chemotherapy and surgically inaccessible, there is an urgent need for the development of novel therapeutic approaches. Our group and others have been evaluating peptide vaccine immunotherapies that target glioma-associated antigens (GAAs). Enhancing the expression of these immunogenic GAAs and MHC I on tumor cells may promote immune-mediated tumor recognition and killing following peptide vaccine immunotherapy. Accordingly, DNA methyltransferase (DNMT) inhibitors have been shown to augment the expression of MHC, tumor antigens, and other immunosensitizing molecules. Guadecitabine (SGI-110), a next-generation DNMT inhibitor prodrug, has been developed to prolong tumor cell exposure to its active metabolite, decitabine. In the current study, we evaluated whether SGI-110 can immunosensitize murine glioma cells to peptide vaccine immunotherapy by enhancing their surface expression of MHC I and a GAA, EphA2. We developed a novel C57BL/6-syngeneic DIPG model by culturing cells from a Sleeping Beauty *de novo* glioma-induced in neonatal mice using a K27M-mutated histone 3.3 plasmid and other oncogenic plasmids (SB-DIPG-11). Flow cytometry analysis showed that SB-DIPG-11 cells express both murine MHC I (H-2Kb/H-2Db) and EphA2 on their surface. *In vitro,* treatment of SB-DIPG-11 and C57BL/6-syngeneic GL261 cells with SGI-110 resulted in a dose-dependent increase of MHC I and EphA2 surface expression. Based on these data, we are evaluating whether SGI-110 can improve the efficacy of peptide vaccine immunotherapy targeting EphA2 *in vivo* using our new DIPG mouse model. Our current data demonstrate that SGI-110 may promote antigen and MHC I expression to improve peptide vaccine immunotherapy for children with DIPG.

IMMU-17. PEPTIDE VACCINE IMMUNOTHERAPY BIOMARKERS AND RESPONSE PATTERNS IN PEDIATRIC GLIOMAS

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Low-grade gliomas (LGGs) are the most common brain tumor affecting children. We recently reported an early phase clinical trial of a peptide-based vaccine, which elicited consistent antigen-specific T cell responses in pediatric LGG patients. Additionally, we observed radiologic responses of stable disease (SD), partial response (PR), and near-complete/complete response (CR) following therapy. To identify biomarkers of clinical response in peripheral blood, we performed RNA sequencing on PBMC samples collected at multiple time points. Patients who showed CR demonstrated elevated levels of T cell activation markers, accompanied by a cytotoxic T cell response shortly after treatment initiation. At week 34, patients with CR demonstrated both IFN signaling and Poly-IC:LC adjuvant response patterns. Patients with PR demonstrated a unique, late monocyte response signature. Interestingly, *HLA-V* expression, before or during therapy, and an early monocytic hematopoietic response were strongly associated with SD. Low *IDO1* and *PD-L1* expression before treatment and early elevated levels of T cell activation markers were associated with prolonged progression-free survival. Furthermore, we identified that genes associated with dendritic cell activation of T-cells correlated with IFNγ ELISPOT counts. Currently we are validating the observed response patterns and biomarkers in high-grade glioma patients treated with peptide vaccine immunotherapy. Overall, our