tumor-bearing mice had an increase in circulating granulocytic MDSC (gMDSC) frequency, and a high gMDSC gene signature correlated with worse outcome of female patients. However, the mechanisms underlying sexual dimorphism of MDSC heterogeneity remain understudied and can provide insights for improved immunotherapy response. Using syngeneic mouse glioma models and sequencing approaches, we show that expression of Y-chromosome-linked genes correlates with upregulation of multiple RNA transcription-related pathways specifically in male mMDSCs. Consistently, adoptive transfer of male mMDSCs but not gMDSCs worsened GBM outcome in male recipients, while the transfer of sex-matched mMDSCs did not impact survival of female mice. In contrast to this cell-intrinsic regulatory pathway, sex steroids had no impact on MDSC profile, as castration or ovariectomy failed to alter MDSC subset accumulation patterns in GBM-bearing mice. Correspondingly, IL-1β, which we had identified as a female-specific drug target, was highly expressed in female but not male gMDSCs. Single-cell sequencing revealed that circulating but not tumor-infiltrating gMDSCs were the primary source of IL-1β and that its neutralization provided a female-specific survival advantage by reducing circulating gMDSCs. This was accompanied by declines in tumor infiltration of microglia, microglia activation status and tumor cell proliferation. In vitro, IL-1β inhibition reduced viability and expression of activation markers by primary microglia. These findings highlight a peripheral gMDSC-microglia communication axis mediated by IL-1β signaling in females with GBM and indicate that expression differences in MDSC subsets represent opportunities for improved immunotherapy efficacy while accounting for sex as a biological variable.

IMMU-11. DUAL TARGETING OF IL-6 AND CD40 OVERCOMES GLIOBLASTOMA RESISTANCE TO IMMUNE CHECKPOINT BLOCKADE

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Glioblastoma (GBM) is refractory to current T cell-based immunotherapies such as checkpoint blockade. GBM is characterized by extensive infiltration of immunosuppressive macrophages (Mφs) that contribute to the treatment resistance. Here we develop a dual-targeting strategy to synergistically activate tumor-associated Mφs, which overcomes GBM resistance to therapeutic blockade of the PD1 and CTLA4 checkpoints. Consistent with a previously established role of IL-6 in alternative Mφ polarization, we show that targeting IL-6 by genetic ablation or pharmacological inhibition moderately improves T cell infiltration and enhances animal survival in a genetically engineered mouse GBM model. However, IL-6 inhibition does not synergize PD-1 and CTLA-4 blockade in GBM. Interestingly, we reveal that anti-IL-6 therapy reduces CD40 expression in GBM-associated Mφs. Our transcriptome analysis identifies a Stat3/HIF-1a-mediated axis, through which IL-6 regulates CD40 expression in Mφs. Finally, we show that combination of IL-6 blockade with CD40 stimulation robustly reverses Mφ-mediated tumor immunosuppression, enhances T cell infiltration, and sensitizes GBM to PD-1 and CTLA-4 blockade treatment, cumulating in inhibited tumor growth and extended animal survival. These findings illustrate a cellular mechanism that regulates Mφ-mediated tumor immunity, and suggest that dual-targeting IL-6 and CD40 may offer exciting opportunities for improving immunotherapy against GBM.

IMMU-13. EFFICACY OF CXCR6 BLOCKADE AS A POTENTIATOR OF ANTI-PD-1 THERAPY FOR THE TREATMENT OF GLIOBLASTOMA

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Glioblastoma (GBM) is an aggressive primary tumor of the brain with a dismal prognosis for patients. Despite the standard of care treatment, median survival is 12–15 months. The blockade of inhibitory checkpoints such as PD-1 and CTLA-4 has become the mainstay immunotherapy to treat solid tumors but it lacks efficacy in treating GBM patients. Emergence of alternative checkpoints on T cells as a mechanism of acquired resistance is considered one of the major hurdles for the success of anti-PD-1 therapy in GBM. Using an orthotopic mouse model of GBM, we have seen that cytotoxic T cells infiltrating the tumor show a preponderance of the chemokine receptor CXCR6 on exhausted cells. Furthermore, ablating CXCR6 along with anti-PD-1 therapy greatly improved anti-tumor immune response. Whereas PBS treated and CXCR6 KO mice had no long-term survivors 40 days post-tumor implantation, 90% of anti-PD-1 treated CXCR6 KO mice were long-term survivors, compared with 12% among anti-PD-1 treated wildtype mice. This supports our hypothesis that blockade of CXCR6 licenses anti-PD-1 blockade by alleviating acquired resistance to anti-PD-1 therapy. We have observed CXCR6 expression on exhausted T cells of GBM patients, making it a promising target for dual therapy with anti-PD-1 in clinical trials.

IMMU-14. ONCOLYTIC ADENOVIRUS DELTA-24-RGD ENGINEERED TO EXPRESS 4-1BBL AS A THERAPEUTIC APPROACH FOR DIPG

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Despite the advances in our understanding of pediatric diffuse midline gliomas (DMG) they remain the leading cause of pediatric death caused by cancer. Our group has demonstrated that the administration of Delta-24-RGD in DIPG models is safe and therapeutically efficacious. An on-going clinical trial with this virus has proven to be safe for these patients. To further improve the anti-tumoral response of the virus, we armed Delta-24-RGD with 4-1BBL (Delta-24-ACT). 41BB is a costimulatory receptor that promotes the survival and expansion of activated T cells, and the generation and maintenance of memory CD8+ T cells. Here, we showed that *in vitro* Delta-24-ACT can infect and express 4-1BBL in murine and human DIPG cell lines. Importantly, 4-1BBL expression in DIPG cell lines was able to activate lymphocytes and increase their IFN-gamma production. In addition, Delta-24-ACT triggered immunogenic cell death in DIPG cell lines, as shown by the release of DAMPs such as ATP, HMGB1, Hsp90a and calreticulin translocation. Delta-24-ACT administration in orthotopic DIPG models was well tolerated and safe. We confirmed the expression of the ligand within the tumor. Moreover, using flow cytometry and multispectral immunohistochemistry we observed profound changes in the tumor microenvironment with an increase in the T-cell populations and a remodeling of the myeloid component before and after treatment. Functional studies showed no differences in lymphocytes isolated from mice treated splenocytes but uncovered TILS that showed a significantly increased in the production of IFN-gamma. Delta-24-ACT treatment of mice bearing orthotopic DIPG murine tumors resulted in a significant increase in the median survival and led to free of disease long-term survivors. In summary, Delta-24-ACT is a virus that builds in our clinical experience with Delta-24-RGD in DIPG patients going a step further to boost the antitumor effect of viral therapy while maintaining a safe profile.

IMMU-15. HEPARIN INHIBITS THE EXTRACELLULAR VESICLE-MEDIATED INDUCTION OF IMMUNOSUPPRESSIVE MONOCYTES IN GLIOBLASTOMA

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Glioblastoma (GBM) is the most common and fatal primary brain tumor in adults. The development of novel therapies is critical, as little has changed regarding the standard of care in nearly two decades. Immunotherapy holds much promise, as treatments including chimeric antigen receptor (CAR) T cells and immune checkpoint blockade inhibitors have transformed the treatment of a number of cancers in recent years. However, GBM patients exhibit profound immunosuppression, limiting the efficacy of these therapies. Understanding the mechanisms of GBM-mediated immunosuppression is critical to overcoming this barrier. GBM-derived extracellular vesicles (EVs) have been shown to mediate the induction of immunosuppressive monocytes, which may point to a mechanism of immunosuppression. EVs make initial contact with target cells through interactions between heparan sulfate proteoglycans, and soluble heparin has been shown to inhibit these interactions in some models. We demonstrate that soluble heparin inhibits the binding of GBM-derived EVs to monocytes in a dose-dependent manner, and that heparin treatment reduces the induction of immunosuppressive monocytes upon *in vitro* conditioning of monocytes with GBM-derived EVs ($p< 0.01$). Further, we demonstrate that heparin treated EV-conditioned monocytes are functionally less immunosuppressive than untreated EV-conditioned monocytes as measured by T cell proliferation in co-culture studies ($p< 0.05$). Taken together, these findings underscore the import of tumor-derived EVs in immunosuppression in GBM, and demonstrate the feasibility of targeting EV-monocyte interactions in treating GBM-mediated immunosuppression.

IMMU-16. TWO DISTINCT SUBSETS OF NATURAL KILLER CELLS ARE ENRICHED IN THE TUMOR MICROENVIRONMENT AND CORRELATE WITH SURVIVAL OUTCOME IN HUMAN GLIOBLASTOMA.

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