marrow in both murine and human samples. This is the first report of significant differences in immune cell populations between different skeletal locations. However, the functional significance of these differences has yet to be determined.

IMMU-07. TUMOR STROMA–TARGETING ANTIBODY-CYTOKINE CONJUGATES TO CONVERT THE IMMUNOLOGICALLY COLD GLIOMA MICROENVIRONMENT INTO A HOT ONE

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Glioblastoma is an immunological "desert". The administration of pro-inflammatory cytokines could shift the balance between tumor-associated immune suppression and anti-tumor immunity but the systemic administration of therapeutically active doses of pro-inflammatory cytokines is hampered by toxic side effects. We investigated different antibody-cytokine fusion products that enable a targeted delivery of interleukin (IL-) 2, IL-12 or tumor-necrosis factor (TNF)α to the tumor site upon systemic administration by binding to a tumor-specific epitope of fibronectin. We confirmed the target antigen expression in *ex vivo* sections of orthotopic syngeneic glioma mouse models and human glioblastoma samples and characterized the distribution of these antibody-conjugates in glioma-bearing mice upon systemic administration using *in vivo* imaging. Subsequently, we demonstrated potent anti-tumor activity of these antibody-fusion proteins in fully immunocompetent orthotopic mouse glioma models and characterized their mode of action. We also translated this immunotherapeutic strategy to treat patients with recurrent glioblastoma systemically with an antibody-fusion protein that enables the targeted delivery of TNFα to the tumor site. This was well tolerated, led to a treatment-associated tumor necrosis and increased the number of tumor-infiltrating T cells. This work builds a basis for future studies with antibody-cytokine fusion proteins as a promising treatment strategy for central nervous system tumors.

IMMU-08. MODELING UPFRONT GLIOBLASTOMA SURGICAL RESECTION AND STEROID USE REVEALS IMMUNOSUPPRESSIVE CHANGES AND SUGGESTS THAT PERIPHERAL LYMPHOCYTE COUNTS ARE ASSOCIATED WITH TUMOR VOLUME AND PROGNOSIS

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Glioblastoma (GBM) and its treatment produces systemic immunosuppression, which is being targeted by immunotherapies. However, it remains unclear how surgical resection and steroids specifically in GBM alter the immune system. To further explore this issue, immunocompetent C57Bl/6 mice were intracranially inoculated with syngeneic glioma cells (GL261 and CT-2A) and growth of tumors was evaluated by MRI. Host immune cell populations were analyzed during surgical resection and steroid administration. Mice with surgically resected tumors had a longer median survival compared to mice subjected to tumor biopsies, and had increased bone marrow sequestration of both CD4 and CD8 T cells with corresponding decreased blood lymphocytes. Furthermore, physiologic doses of dexamethasone administered perioperatively decreased tumor edema, but increased the number and proliferative capacity of both marrow and circulating MDSCs while generating no survival benefit. Independent of therapy or dexamethasone, intracranial tumor volume correlated linearly with decreased CD4 and CD8 T cells in peripheral blood, and increased T cell sequestration within the bone marrow. We validated these parameters in steroid-naïve newly diagnosed GBM patients and observed decreased lymphocytes correlated linearly with increased tumor volume. When initial lymphocyte counts in both steroid-naïve and steroid-administered patients were used in univariate and multivariate models predicting progression-free survival and overall survival, decreased initial lymphocyte counts were an independent predictor of decreased progression free survival and decreased overall survival, with steroid use and initial tumor size falling out of significance during stepwise selection. Taken together, tumor volume is linearly correlated with marrow sequestration of lymphoid cells, but both surgery and steroid administration further suppress active immune responses along lymphoid and myeloid lineages. Furthermore, decreasing peripheral lymphocyte counts at diagnosis of GBM indicate an immune system less able to mount responses to the tumor and portent a worse progression free and overall survival.

IMMU-09. CONCURRENT DEXAMETHASONE LIMITS THE CLINICAL BENEFIT OF IMMUNE CHECKPOINT BLOCKADE IN GLIOBLASTOMA

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BACKGROUND: Dexamethasone, a uniquely potent corticosteroid, is frequently administered to brain tumor patients to decrease tumor-associated edema, but limited data exist describing how dexamethasone affects the immune system systemically and intratumorally in glioblastoma patients – particularly in the context of immunotherapy. METHODS: We evaluated the dose-dependent effects of dexamethasone when administered with anti-PD-1 and/or radiotherapy in immunocompetent C57BL/6 mice with syngeneic GL261 or CT-2A glioblastoma tumors, including analyses of intracranial tumors, draining lymph nodes, and spleen. Clinically, the effect of dexamethasone on survival was additionally evaluated in 181 consecutive IDH-wildtype glioblastoma patients treated with anti-PD-(L)1, with adjustment for relevant prognostic factors. RESULTS: Despite the inherent responsiveness of GL261 to immune checkpoint blockade, concurrent dexamethasone administration with anti-PD-1 therapy decreased survival in a dose-dependent fashion and decreased survival following anti-PD-1 plus radiotherapy in both GL261 and immunoresistant CT-2A models. Dexamethasone quantitatively decreased T lymphocytes by reducing the proliferation while increasing apoptosis. Dexamethasone also decreased lymphocyte functional capacity. Myeloid and NK cell populations were also generally reduced. Thus, dexamethasone negatively affects both the adaptive and innate immune responses. As a clinical correlate, a retrospective analysis of 181 consecutive IDH-wildtype glioblastoma patients treated with PD-(L)1 blockade revealed worse survival among those on baseline dexamethasone. Upon multivariable adjustment with relevant prognostic factors, baseline dexamethasone use – regardless of dose – was the strongest predictor of poor survival (reference no dexamethasone; < 2mg HR 2.28, 95%CI=1.41–3.68, p=0.001; ≥2mg HR 1.97, 95%CI=1.27–3.07, p=0.003). CONCLUSIONS: Our preclinical and clinical data indicate that concurrent dexamethasone therapy may be detrimental to immunotherapeutic approaches for glioblastoma patients. Our preclinical analyses also suggest that dexamethasone's detrimental effects are dose-dependent, suggesting that the lowest possible dose should be used for patients when dexamethasone use is unavoidable. Careful evaluation of dexamethasone use is warranted for neuro-oncology patients undergoing immunotherapy clinical trials.

IMMU-10. GENOMIC DIFFERENCES UNDERLIE MYELOID-DERIVED SUPPRESSOR CELL SEXUAL DIMORPHISM IN GLIOBLASTOMA

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A potently immunosuppressive tumor microenvironment facilitates progression of glioblastoma (GBM). We previously demonstrated that myeloid-derived suppressor cell (MDSC) subsets promote tumorigenesis in a sex-specific manner, contributing to sexual dimorphism in GBM incidence and prognosis. Our findings indicated that proliferating monocytic MDSCs (mMDSCs) accumulate in tumors of male mice and patients, while female

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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