tors. While the immunosuppression in GBM is severe, a causative link between each facet of immunosuppression and overall survival is lacking. We used two strategies to block T-cell sequestration into the bone marrow and evaluated the extent survival was impacted in experimental GBM. First, we evaluated the extent a novel and off-the-shelf combination immunotherapy that uses extended 1/2-life IL-2 and anti-PD-1 reverses bone marrow T-cell sequestration. Sham treatment or anti-PD1 monotherapy did not alter T-cell sequestration in the bone marrow and animals had no enhanced survival. Extended 1/2-life IL-2 monotherapy and combination strategy both prevented T-cell sequestration into the bone marrow. However, only combined therapy, which also prevented MHC class II downregulation, improved survival. Second, we determined that glioma-bearing adrenalectomized mice do not present with bone marrow T-cell sequestration. However, sera of glioma-bearing adrenalectomized mice is as immunosuppressive as glioma-bearing controls. Blocking bone marrow T-cell sequestration in the presences of serum immunosuppression led to no survival benefit in glioma-bearing adrenalectomized mice compared to controls. In short, bone marrow T-cell sequestration alone does not correspond with overall survival in experimental glioma. Importantly, a concerted effort to reverse MHC class II downregulation and define inhibitory circulating factors may have the highest impact in immunotherapeutic efficacy and improving patient survival.

IMMU-20. HYDROGEL-CXCL9 VACCINE RESULTS IN MRNA DELIVERY TO DENDRITIC CELLS AND POTENT ANTI-TUMOR RESPONSES IN GBM

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INTRODUCTION: Our group and others have shown mRNA-vaccines have significant anti-tumor efficacy in the treatment of brain tumors and are currently being tested in first-in-human trials. To further enhance mRNA delivery, a hydrogel platform was developed with the addition of CXCL9 to promote immune cell trafficking. METHODS: We generated the vaccine by utilizing a hydrogel platform. CXCL9 and Nano-mRNA were loaded into the hydrogel. *In vitro* recruitment of DCs and NK was evaluated by fluorescence microscopy. *In vivo* recruitment of immune cells was analyzed by flowcytometry after collecting the fat pad, spleen, and tumor samples from KR158b-luc and Gl261-gp100 tumor-bearing animals 3, 5 and 10 days after vaccine delivery. The efficacy of the vaccine was evaluated by measuring overall survival, and tumor growth was measured by IVIS live-imaging. RESULTS: Dendritic cells(DCs) and natural killer(NK) cells were able to efficiently migrate within the hydrogel-CXCL9 platform and uptake and express mRNA in vivo. In vitro, the hydrogel-CXCL9 was combined with nanoparticles loaded with total tumor RNA, and the vaccine was delivered to KR-158-luc and GL261-gp100 tumor-bearing animals via mammary fat pad SQ injection. Flow cytometry of the fat pad and draining lymph nodes demonstrated showed significant recruitment of endogenous DCs including inflammatory-DC(P= 0.0035), conventional-DC1(P= 0.0076), pDC(P=0.0028), NK(p= 0.0025 compared to the control group. In two different tumor models, a single dose of the vaccine resulted in significant survival benefits compared to control animals (n=10,P< 0.0001). SQ injection was superior to intracranial injection of the vaccine. Vaccinated animals showed an increased number of antigen-specific CD8 T cells in spleen(P= 0.0001) and tumor-microenvironment(P= 0.0070). CONCLUSION: The hydrogel-CXCL9 platform results in efficient delivery of mRNA loaded nanoparticles to endogenous DCs and also causes an upregulation of NK cells with resultant improved survival in murine GBM models including the highly resistant KR158 model with a single dose. Further studies are ongoing.

IMMU-21. DIFFERENTIAL EXPRESSION OF ADHESION MOLECULES DEFINES MYELOID CELL INFILTRATION IN GLIOBLASTOMA AND COMPRISES A THERAPEUTIC TARGET

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A potently immunosuppressive tumor microenvironment facilitates progression of glioblastoma (GBM). Immunotherapies have had variable success in improving the outcome of GBM patients, suggesting that there is a need to gain insight into the mechanisms of immunosuppression. Myeloid-derived suppressor cells (MDSCs) associate with poor prognosis and treatment resistance of GBM patients, but the distinct role of individual populations is not well-defined. We previous showed that monocytic MDSCs (mMDSCs) accumulated in tumors of mice and patients, while granulocytic MDSCs (gMDSCs) mainly remained in the circulation. Furthermore, nonspecific targeting of mMDSCs with chemotherapies provided therapeutic benefit in preclinical models of GBM, suggesting that mMDSCs drive disease progression. To investigate the differential function of mMDSCs versus gMDSCs in GBM, we adoptively transferred bone marrow-derived MDSC subsets into tumor-bearing mice. Mice that received mMDSCs succumbed to disease sooner compared to control mice, which was not observed with gMDSC transfer. To determine the basis of this pro-tumorigenic activity of mMDSCs, we performed ATAC-sequencing and comparison of differentially accessible regions indicated that cell adhesion pathways were significantly upregulated in mMDSCs. Aligned with this epigenetic profile, mMDSCs from bone marrow and blood had significantly higher surface integrin β1 and integrin β7 expression compared to gMDSCs. To evaluate the role of integrins in MDSC behavior, we pre-treated mMDSCs with anti-integrin β1 prior to adoptive transfer. Blockade of integrin β1 interfered with the pro-tumorigenic role of mMDSCs compared to isotype controls. Similarly, blockade of integrin β1 and integrin β7 systemically extended the survival duration of tumor-bearing mice. Finally, high expression of integrin β1 and integrin β7 served as a poor prognostic indicator in GBM patients. Our findings indicate that modulation of immunosuppressive myeloid cells by leveraging differences in adhesion mechanisms represents a potential immunotherapeutic option for GBM.

IMMU-22. THE IMPACT OF MICROGLIA MODULATION ON GBM PROGRESSION IN SYNGENEIC MOUSE MODELS

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BACKGROUND: The tumor immune microenvironment (TME) of Glioblastoma consists of almost myeloid-derived macrophages and microglia called TAMs. We have shown that the disruption of CD47-Sirpα-axis induces an antitumor activity of TAMs against GBM in immune-deficient mice, through increases of phagocytosis of tumor cells by TAMs. We have aimed to study the role of microglia and its activation/depletion on GBM progression, in the syngeneic GBM model in immune-competent mice. We have studied the interplay of innate and adaptive immune response after activation and depletion of microglia and the effect on tumor progression and outcome of the mice. MATERIAL AND METHODS: We used different colonies of genetically modified immunocompetent mouse strains to genetically activate/deplete microglia in the tumor context. We generated *Sall1CreERT2/fl* mice and Cre-negative littermates. The application of Tamoxifen in this constellation leads to the excision of the transcription factor Sall1 and subsequent enhanced microglia activity. Conversely, we generated *Sall1CreERT2 x Csf1r fl/fl* animals and the respective heterozygous and Cre-negative littermates in which Tamoxifen treatment leads to inactivation of microglia through the deletion of Csf1r. Glioblastoma tumors were induced by intracerebral injection of GL261, CT2A, or retrovirus-induced PDGF-Akt in pups and Tamoxifen treatment was started once the tumors were detected. RESULTS: We observed a survival advantage in tumor-bearing mice after activation of microglia in *Sall1CreERT/fl* animals compared to Cre-negative littermates. Genetic depletion of microglia in this model resulted in a shorter lifespan in microglia-depleted animals compared to Cre-negative littermates. Furthermore, the iTME in these tumors is subjected to scRNAseq analysis to identify mechanistic insights. CONCLUSION: Microglia are important players in tumor development and progression of glioblastoma in mouse models. These cells may be targeted in future immunotherapeutic approaches for patients.

IMMU-23. NEOANTIGEN-DIRECTED CELLULAR THERAPY IN A PRECLINICAL MOUSE MODEL OF MALIGNANT GLIOMA

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Adoptive cellular therapy in the form of CAR T cells or TCR engineered T cells has emerged as a novel approach in the treatment of both solid and hematologic malignancies. Neoantigens generated by tumor somatic mutations represent potentially attractive therapeutic targets in this context owing to their tumor-specific expression and circumvention of immunological tolerance. However, existing cell therapy systems generally target self-proteins or virally overexpressed antigens that fail to recapitulate the features of endogenous tumor neoantigens. Thus, there exists a need for a model in which tumor-specific neoantigens can be targeted via adoptive cellular therapy. Prior work from our lab identified the $Imp3_{D81N}$ mutation (mImp3) within GL261 as a neoantigen recognized by CD8 T cells in both intracranial tumors and draining cervical lymph nodes. To generate a system for targeting this neoantigen, we isolated and cloned mImp3-specific TCRs through a single-cell sort followed by a nested multiplexed PCR reaction. The specificity and functionality of these isolated TCRs