OTME-16. POLIO VIROTHERAPY OF MURINE BRAIN TUMORS CAUSES MICROGLIA/MACROPHAGE PROLIFERATION AND INFLAMMATION THAT IS POTENTIATED BY IMMUNE CHECKPOINT BLOCKADE
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PVSRIPo is a novel viral immunotherapy that has shown evidence of efficacy in a phase I clinical trial for recurrent GBM, resulting in 21% survival rate at 36 months following treatment. To improve clinical response rate, it is critical to resolve the mechanisms of action and therapy resistance in vivo, thereby designing effective combination therapy strategies. We used immunocompetent mouse models of glioma (CT2A) and metastatic melanoma (B16) to dissect early and late events following virotherapy with PVSRIPo. A blinded systematic review of the pathology from 62 intracranial tumors, collected on different days following PVSRIPo (or control) treatment, was performed. An overall treatment effect, measured by tumor shrinkage, dis-cohesive growth pattern, microgliation, enrichment, was present in 88% of tumors on day 8, but the tissue response rate fell to 42% on days 10 & 12, and 14% on day 15. The control group showed no treatment effect throughout. RNASeq from the same set of samples showed acute induction of type-I interferon-related inflammation that faded with time in Gene Set Enrichment Analysis. This suggests that sustaining adaptive antitumor immunity elicited by immediate-early intratumor IFN-DNK dominant inflammation is critical to long-term remission. Careful review of the post treatment pathology revealed an early enrichment of both T cells and microglia in the tumor microenvironment with a high Ki-67 proliferation index. We propose that the PVSRPo therapy effect is dependent on macrophage/microglia mediated cellular immune response, likely in response to direct viral infection. This suggests potential therapeutic interventions, including blockade of the PD1/PD-L1 immune checkpoint, to potentiate antitumor CD8+ T cells in response to PVSRPo therapy. Indeed, combination therapy with αPD-L1 antibody in the CT2A model showed higher long term remission (37%, n=11), compared to either monotherapy; this effect is CD8+ T cell- and macrophage-dependent, demonstrated by depletion studies in vivo.

OTME-17. SINGLE CELL CHARACTERIZATION OF THE IMMUNE MICROENVIRONMENT OF MELANOMA BRAIN AND LEPTOMENINGEAL METASTASES
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Melanoma brain metastasis (MBM) and leptomeningeal metastases (LMM) are two manifestations of melanoma dissemination to the CNS with vastly different survival outcomes. Analysis of single cell RNA-Seq data from 43 clinical specimens has uncovered a distinct, immune-suppressed T cell landscape in the LMM microenvironment that is distinct to those of the brain and skin metastases. An LMM patient with an extraordinarily long survival and documented response to therapy demonstrated an immune repertoire that was distinct from those of typical poor survivors and more similar to those from non-LMM donors. Analysis of 155 single cells in 24 human GBM samples demonstrated differences in melanoma cells and macrophages, coupled with increased levels of T cells and dendritic cells in the CSF of the extraordinary responder, whereas poor survivors showed no improvement in T cell responses. In MBM patients, targeted therapy and immunotherapy was associated with increased immune infiltration, with similar T cell transcriptional diversity noted between skin metastases and MBM - suggestive of immune cell trafficking into the brain. Treatment with targeted therapy was associated with an enrichment of CD8 T cells. Immunoabscence was associated with a more diverse immune landscape and higher numbers of antibody-producing cells. These findings were confirmed by multiplexed staining of patient specimens and using an immune-competent mouse model of MBM. Correlation analysis across the entire immune landscape identified the presence of a rare, novel population of dendritic cells (DC3s) to be correlated with increased overall survival, regardless of disease site/treatment. The presence of DC3s positively regulated the immune environment of both patient samples and preclinical melanoma models through modulation of aIL27 mediated MHC expression in tumor cells. Our study indicates the first comprehensive atlas of two distinct sites of melanoma CNS metastases and identifies rare populations of cells that underlie the biology of this devastating disease.

OTME-18. TARGETED CRISPR/CAS9 GENE-EDITING REGULATES THE BRAIN TUMOR ENVIRONMENT
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Glioblastoma multiforme (GBM) is the most common malignant brain tumor. Recent immunotherapy has demonstrated potential to treat GBM. However, the immune suppressive tumor environment in the brain represents a significant barrier for the treatment of GBM. Overexpression of programmed death ligand-1 (PD-L1) in GBM tumor cells and macrophages plays a key role in GBM vitality, proliferation, and migration, while also suppressing the immune system. We developed a CRISPR/Cas9 gene-editing system to delete whole cell PD-L1. Human PD-L1 targeted sgRNA were cloned into CRISPR/Cas9 plasmids with or without an HDR template. CRISPR/Cas9 were treated to human GBM U87 cells for 15, 30, 60, 120 and 240 minutes. The intracellular concentration of CRISPR/Cas9 exhibited a time-dependent increases. A GFP tagged CRISPR/Cas9 plasmid was developed to test the transfection efficacy. Higher levels of GFP+ U87 cells were observed at day 3. CRISPR/Cas9 showed a greater PD-L1 knockout at day 3. The PD-L1 reduction limited the proliferation of U87 cells. A scratch assay showed that PD-L1 deletion inhibited the migration of U87 cells. An in vitro GBM model was developed by co-cultivation of U87 cells and macrophages. CRISPR/Cas9 treated co-cultures changed the ratios of U87 cells and macrophages and polarized tumor associated macrophages (TAM) from M2 toward M1. CRISPR/Cas9 gene-editing effectively depleted PD-L1 in U87 cells. Successful deletion of PD-L1 prevented U87 cell growth and migration, and altered the TAMs plasticity and the tumor environment.

OTME-19. REGULA REGULATION OF GLOMAGENESIS AND STEMNESS THROUGH ACID SENSOR ASIC1A
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Glioblastoma multiforme (GBM) is the most prevalent and aggressive type of adult gliomas. Despite intensive therapy including surgery, radiation, and chemotherapy, invariable tumor recurrence occurs, which suggests that glioblastoma stem cells (GSC) render these tumors persistent. Recently, GSC differentiation has emerged as an alternative method to treat GBM, and most of current studies aim to convert GSC to neurons by a combination of transcriptional factors. As the tumor microenvironment is typically acidic due to increased glycolysis in tumor cells, here, we explored the role of acid-sensing ion channel 1a (ASIC1a), an acid sensor, as a tumor suppressor in gliomagenesis and stemness. The bioinformatics data from TCGA shows that ASIC1a expression levels in GBM tumor tissues were lower than those in normal brain, and glioma patients with elevated ASIC1a expression have longer survival than those with lowered ASIC1a expression. Our immunohistochemistry data from tissue microarray shows that ASIC1a expression is negatively correlated with glioma grading. Functional studies reveal that the downregulation of ASIC1a promotes glioma cell proliferation and invasion, while upregulation of ASIC1a inhibits their proliferation and invasion. Furthermore, ASIC1a suppresses glioma cells’ growth and proliferatation through G1S arrest and apoptosis induction. Mechanistically, ASIC1a negatively modulates glioma stemness via inhibition of the Notch signaling pathway and GSC markers CD133 and ALDH1. Our findings indicate that ASIC1a is a tumor suppressor in gliomagenesis and stemness and may serve as a promising diagnostic biomarker and target for GBM patients.

OTME-20. CHITINASE-3-LIKE-1 (CH3L1) PROTEIN COMPLEXES REGULATE THE IMMUNOSUPPRESSIVE MICROENVIRONMENT IN GLOIOBLASTOMA
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Glioblastoma (GBM) is the most common and highly malignant brain tumor in adults. Despite advances in multimodal treatment, GBM re-

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mains largely incurable. While immunotherapies have been highly effective in some types of cancer, the disappointing results from clinical trials for GBM immunotherapy represent continued challenges. GBM is the most common and most aggressive type of glioma, and is associated with a high degree of immunosuppression by the tumor microenvironment (TME). However, understanding the mechanisms of immune evasion by GBM remains elusive. Based on unbiased approaches by Gao et al. that Chitinase 3-like protein 1 (CHI3L1), a previously known as human homolog YKL-40, is highly expressed in GBM, which is regulated by the CHI3L1–PI3K/AKT/mTOR signaling in a positive feedback loop. Gain- and loss-function studies reveal that CHI3L1 plays a predominant role in regulating an immunosuppressive microenvironment by recruiting tumor-associated macrophages (TAMs). Using the liquid chromatography-mass spectrometry and orthogonal structure-based screening, we found that Galectin-3 binding protein (Gal3BP) and its binding partner, Galectin-3 (Gal3), interact competitively with the same binding motif on CHI3L1, leading to selective migration of M2-like versus M1-like bone marrow-derived macrophages (BMDMs) and resident microglia (MGs). Mechanistically, the CHI3L1-Gal3 protein complex governs a transcriptional program of NFκB/C/EBPβ to control the protumor phenotype of BMDMs, leading to inhibition of T cell infiltration and activation in the GBM TME. However, Gal3BP can reverse CHI3L1-Gal3 induced signaling pathway activation and subsequent protumor phenotype in TAMs. Based on protein binding motifs, a newly developed Gal3BP mimic peptide can attenuate immune suppression and tumor progression in the syngeneic GBM mouse models, including decreasing M2-like TAMs and increasing M1-like TAMs and T cell infiltration. Together, these results shed light on the role of CHI3L1 protein complexes in immune evasion by glioblastoma and as a potential immunotherapeutic target for this devastating disease.

OTME-21. THE ROLE OF GLIOBLASTOMA ASSOCIATED MESENCHYMAL STEM CELLS IN IMMUNE SUPPRESSION
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Glioblastoma (GBM) is an aggressive brain cancer with, an overall survival of 14.6 months. The tumor microenvironment in GBM plays major roles in immunosuppression and modulation of the response to therapies. GBM patients with higher levels of mesenchymal stem cells (G-MSC) show poor overall survival as compared to patients with lower G-MSC levels. Our lab found that levels of G-MSC correlate with CD4+ T cells in humans and murine models of GBM, and with immunosuppressive molecules like PTG2, the gene for cyclooxygenase 2. To investigate the mechanism by which G-MSCs promote immunosuppression, we isolated G-MSCs from an orthotopic mouse model of GBM and subjected them to RNAseq analysis to obtain an unbiased picture of transcriptomic changes occurring upon activation. We identified changes in multiple immune modulating pathways important for antigen presentation, leukocyte migration and activation, and immune checkpoints. Our findings indicate that G-MSCs represent a key immune modulating factor in the microenvironment. Further dissection of the role of these cells in immune modulation will aid us in understanding the biology of the brain tumor microenvironment and identifying potential combination therapies.

OTME-22. BIOINFORMATICAL EVALUATION OF ECM MOLECULES AND ANGIOGENIC ASSOCIATE GENES IN DIFFUSE MALIGNANT GLIOMA (DMG): MAPPING THE TUMOUR MICROENVIRONMENT
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Paediatric Diffuse Intrinsic Pontine Glioma (DIPG) is a devastating cancer of an extremely aggressive nature, located in the pontine area of the brain. DIPG primarily affects children, with the average age of diagnosis between 6 and 7 years. Unfortunately, the outlook and overall survival remains bleak. We have found that immunosuppressive genes are central to and drive DIPG growth; there remains several gaps in understanding the DIPG microenvironment landscape. The focus of this study is to begin to examine mRNA expression of genes associated with blood vessel development, angiogenesis, and extracellular matrix molecules (ECM) in normal brain and DIPG by using publicly available gazing human glioblastoma superset databases. In-depth bioinformatics from GSE26576 dataset included differential expression and gene ontology (GO) with KEGG pathway analyses using Gene Expression Omnibus (GEO) and DAVID, which have revealed a number of immunosuppressive genes that may affect DIPG angiogenesis processes (p<0.05). 38 of such genes from 9 different GO terms were then included in a protein-to-protein interaction network that revealed a surprising connection between MMP16, CSGP4 and COL11A1. Subsequently, using R2 genomic visualisation platform from publicly available single cell RNAseq data we showcased the difference in their individual expression based on the molecular subtypes of DIPG histone 3 (H3) mutation (K27M, wild type and K27Q) with a strong statistical significance (p<0.05). Interestingly, during normal paediatric development such genes showed consistent expression, suggesting their potential complications in DIPG angiogenesis. Overall, this bioinformatic approach has led to the identification of a set of immunosuppressive genes that further understanding and future research in DIPG will add to the documentation of the host/tumour microenvironment landscape and our plan is to continue to explore this map to spatial and temporal expression of these genes.

OTME-23. SINGLE-CELL TRANSCRIPTIONAL AND EPIGENOMIC IMMUNE LANDSCAPE OF BREAST DEHYDROGENASE STRATIFIED HUMAN GLIOMAS
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The brain tumor immune microenvironment (TIME) continuously evolves during glioma progression, but a comprehensive characterization of the gloma-centric immune cell repertoire beyond a priori cell states is uncharted. In this study, we performed single-cell RNA-sequencing (scRNA-seq) and single-cell Assay for Transposase-Accessible Chromatin using sequencing (sc-ATAC-seq) on ~100,000 tumor-associated immune cells from seventeen isocitrate dehydrogenase (IDH) mutation classified primary and recurrent human gliomas and non-glioma brains (NGBs). Our analyses revealed sixty-two transcriptionally distinct myeloid and lymphoid cell states within and across glioma subtypes and we noted an increasing disease severity concomitant with invading monocyte-derived cells and lymphocytes. Specifically, certain microglial and monocyte-derived subpopulations were associated with antigen presentation gene modules, akin to cox-presenting dendritic cells (DCs). We identified cytotoxic T cells with poly-functional cytolytic states mostly in recurrent IDH-wt gliomas. Furthermore, ligand-receptor interaction analyses showed a preponderance of antigen presentation and phagocytosis over the checkpoint axis in IDH-wt compared to IDH-mut gliomas. Additionally, our sc-ATAC-seq analyses revealed differences in regulatory networks in NGBs, IDH-mut and IDH-wt glioma associated immune cells. In particular, we noted abundant usage of inflammatory transcription factors (TFs) as exemplified by Nuclear factor kappa B and Activator Protein-1 TF family in IDH-wt microglia when compared with microglia from IDH-mut and NGBs. Unique features such as amplification of 11- Zinc Finger Protein accessibility were restricted to monocyte derived cells and were not observed in microglia. Finally, sc-ATAC-seq profiles of CD8+ exhausted T cells from IDH-wt showed strong enhancer accessibility on Cytotoxic T lymphocyte-associated protein 4, Lymilin and Hepatitis A Virus Cellular Receptor 2 but no enrichment on PDCD1 (gene encoding Programmed cell death protein 1) was seen. In summary, our study provides unprecedented granular detail of transcriptionally defined glioma-specific immune contexture that can be exploited for immunotherapy applications.

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