Title

IMMU-07. IMMUNE PROFILES IN THE SAN FRANCISCO ADULT GLIOMA STUDY (AGS) USING IMMUNOMETHYLOMICS

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intracranial tumors were refractory. CyTOF analysis revealed that MHC-II+ intratumoral macrophages were twice as frequent in subcutaneous versus intracranial tumors. Similarly, dendritic cells were abundant in subcutaneous tumor-draining lymph nodes, but absent in intracranial tumor-draining (cervical) lymph nodes. Mice previously cured of subcutaneous tumors rejected intracranial tumors upon rechallenge. Taken together, these results suggest that subcutaneous tumors allow superior antigen presentation, resulting in priming and sustained immunological memory after CPI. We then investigated FLT3-ligand, an essential cytokine for dendritic cell maturation, as a therapy to improve antigen presentation in mice with intracranial tumors. We administered recombinant FLT3-ligand intraperitoneally for 10 days after engraftment of intracranial tumors in naive hosts. FLT3-ligand dramatically increased the frequency of dendritic cells and CD8+ T cells in spleens and cervical lymph nodes. Notably, FLT3-ligand monotherapy also caused durable rejection of intracranial tumors in 30% of mice. These results indicate that sufficient T cell priming can confer immunological control of GBM, even in a macrophage-rich, T cell-poor microenvironment.

IMMU-05. LATE EFFECTS OF INTRACRANIAL RADIATION INDUCES RESISTANCE TO IMMUNE CHECKPOINT BLOCKADE THERAPY THAT IS PARTIALLY REVERSIBLE WITH CSF-1R INHIBITION

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Primary and metastatic CNS malignancies remain incurable despite aggressive therapy with surgery and radiation. Immunotherapy has shown promise in many solid and hematologic malignancies, however, results have been disappointing in recurrent primary CNS tumors. Additionally, while upfront immune checkpoint blockade therapy (ICB) has shown equal efficacy intracranially and extracranially, these responses are abrogated in previously irradiated brain lesions. Together, we hypothesize that radiation modulates the brain microenvironment to permit tumor growth and suppress anti-tumor immunity. As such, we developed a mouse model whereby orthotopic transplantation of murine gliomas into previously irradiated normal brain results in a more aggressive tumor phenotype. Moreover, the ICB-sensitive glioma cell line, GL261, is resistant to ICB therapy when implanted into a previously irradiated brain microenvironment. Immunophenotyping revealed a decreased ratio of CD8:CD4 T cells within tumor-infiltrating lymphocytes isolated from previously irradiated mice though the relative frequency of neoantigen-specific CD8 T cells was slightly increased and no difference in PD-1 expression was observed. Alternatively, the frequency of microglia and tumor-infiltrating $CD11b^{+}$ $Gr\text{-}1^{+}$ myeloid-derived suppressor cells (MDSC) was increased following irradiation suggesting a potential role for these myeloid cells in the immunosuppressive effects noted. Consistently, the administration of a CSF-1R inhibitor, which has been shown to reduce the number of microglia and block MDSCs, partially resensitizes GL261 cells to ICB therapy. In summary, we have developed a model that recapitulates the late effects of radiation on immunotherapy-resistance in CNS tumors. Preliminary results suggest that these late radiation effects are mediated through an increased myeloid population, and that inhibition of these cell subsets via the CSF-1R pathway can partially restore efficacy of ICB therapy. Furthermore, this model may provide further insight into additional therapeutic strategies that can be used to overcome these mechanisms of resistance induced by radiation therapy in the CNS.

IMMU-06. ABSENCE OF THE AMINO ACID STRESS-SENSOR GCN2 REDUCES SUPPRESSIVE EFFECTS OF MDSCs IN GLIOMA

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Regulation of nutrient availability is a critical way by which tumors exert their immunosuppressive effects on the host. The glioblastoma microenvironment, in particular, is enriched in expression of indoleamine 2,3- dioxygenase 1 (IDO1), which depletes tryptophan from the microenvironment. Upon depletion of tryptophan, the amino acid stress kinase, general control non-derepressible 2 (GCN2), inhibits general protein synthesis, and upregulates a number of genes to promote the cellular survival. In this study, we explored the effects of a deficiency of the GCN2 pathway on infiltrating immune cells in the context of the glioma. Interestingly after GL-261 (Glioma mouse model cell line) injections in GCN2 knock out (KO), and wild type (WT) mice, GCN2 KO mice survived significantly (p_{value}:0.0066) longer compared to WT mice. *In-vivo* flow cytometric analyses of these mice, showed significant reduction in $CD45^{hi}CD11b^{+}$ (myeloid cell marker) and Ly6C (monocytic MDSC marker) population in GCN2 KO mice. To study the mechanisms responsible for this, we generated *in-vitro* bone-marrow-derived MDSCs from both WT and GCN2KO mice. Surprisingly, the *in-vitro* T-cell suppressive effects of GCN2 KO MDSCs were significantly attenuated, with a decrease in arginase-1 (Arg-1) expression as a potential mechanism for this observation. To validate this phenomena *in-vivo*, we injected GCN2 KO and WT mice with GL-261 tumor cells, and after 18 days post tumor injection, we isolated Gr-1 (pan-MDSC marker) positive cells from tumor bearing brains, and checked for Arg-1 level by RNA and protein. Our data, both by protein and RNA demonstrated significant reduction in Arg-1 levels. Importantly, *ex-vivo* isolated Gr-1 myeloid cells from tumor bearing brain of GCN2 KO and WT mice shows dramatically reduced suppressive capabilities. In conclusion, our data demonstrate the importance of GCN2 pathway on MDSC functionality, and provide insight on how amino acid sensing enzymes may regulate immunity in glioblastoma.

IMMU-07. IMMUNE PROFILES IN THE SAN FRANCISCO ADULT GLIOMA STUDY (AGS) USING IMMUNOMETHYLOMICS

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Glioma patients demonstrate abnormalities in peripheral blood leukocytes that have been associated with survival time. Here we assessed immune cell profiles in archival blood samples obtained 5–25 years ago using a novel epigenetic approach called immunomethylomics. We first validated the approach to measure the proportions of CD4 T cells, CD8 T cells, B cells, NK cells, neutrophils, and monocytes in archival blood using the new 850,000 feature EPIC Illumina methylation bead array. The immunomethylomic assay was shown to match the performance of multiparametric flow cytometry and thus is a highly accurate method for immune profiling. We next measured cell profiles in blood from 312 molecularly defined AGS subjects. In this cohort we enriched for patients with triple negative tumor subtypes (IDH wildtype, 1p19q negative, TERT non-mutant). Elevations in the neutrophil lymphocyte ratio (NLR) significantly increased with grade, whereas the proportions of T cells decreased with increasing grade. Using an established cut point of > 4.0 for the NLR revealed that this immune parameter was associated with shorter survival times in glioblastoma (median overall survival (mOS) 12.6 vs. 16.9 months) and non-glioblastoma (mOS 16.7 vs. 36 months) patients. Ongoing analyses of the cohort will be presented to evaluate the effects of age, gender, surgery, chemoradiation and tumor grade on the survival results. Because DNA based immune cell profiles do not require intact cells nor preserved proteins, they provide a powerful tool to glean potentially important immunologic information from historic stored samples that could not otherwise be utilized using current cytometry-based approaches.

IMMU-08. THE ROLE OF WNT SIGNALING ON T-CELL INFILTRATION IN GLIOBLASTOMA

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BACKGROUND: Glioblastomas (GBMs) are considered immunologically cold tumors, making it difficult to apply immunotherapeutic strategies. Oncogenic pathways intrinsic to the tumor or the microenvironment can influence T-cell infiltration. WNT/β catenin is one such pathway implicated in modulating T-cell infiltration in other solid malignancies. In this study, we examine the influence of WNT signaling on the infiltration of T-cells in GBM. METHODS: Using the TCGA dataset, we analyzed the mRNA expression of both β-catenin dependent (canonical) and independent (non-canonical) WNT ligands and CTNNB1 (β-catenin) in GBM. The expressions of these components were correlated with a T-cell signature (CD3e, CD3d, CD3g, CD4, CD8a, and CD8b) and survival. Additionally, multiple GSC cell lines, derived from human GBM samples, were profiled for different WNT ligands and cells with the highest and lowest expression were selected for further experiments. *In vitro,* we also utilized a T-cell migration assay to measure the effect of WNT ligands (WNT3a and WNT5a) on T-cell migration towards a GL261 monolayer. RESULTS: *In silico* analysis of the TCGA dataset revealed downregulation of most WNTs in GBM and