MB is the most common malignant brain tumor in children and although standard therapy is usually curative in greater than 70% of patients, survivors often suffer sequelae of developmental and neurological side effects. A significant obstacle in treating MB has been the heterogeneous nature of such tumors, encompassing four molecularly distinct subgroups that differ in their clinical presentation, mutational landscape and response to treatment. Immunologic targeting of MB tumors presents an attractive strategy that has shown significant promise in adult brain tumors, demonstrating effective tumor cell killing while minimizing collateral damage to the surrounding brain. Immunohistochemistry and gene expression analyses of human MB samples have given more clarity into the cellular immune infiltrates that populate the tumor microenvironment across subtypes. These studies revealed subgroups of MB could be stratified based on the presence of tumor-associated macrophage and inflammatory gene upregulation, suggesting a potential immunotherapeutic avenue to target tumors based on subtype classification.

We have recently demonstrated that subtypes of murine MB possess distinct immunologic profiles that respond differentially to immune checkpoint blockade in mice bearing syngeneic intracranial MB tumors. Through orthotopic transplantation of tumor cells in C57Bl/6 animals, we adapted two established mouse models recapitulating human SHH-group and Group 3 MB and confirmed their maintenance of subtype-specific gene expression following serial in vivo passaging. We observed that murine SHH-group tumors were characterized by being more infiltrated with immune cells, with significantly higher proportions of lymphocytes and myeloid cells, including myeloid-derived suppressor cells (MDSCs) and tumor-associated macrophages (TAMs). However, when we measured immunosuppressive subsets of CTLA-4 or PD-1 expressing T cells, murine Group 3 tumors contained higher percentages of PD-1+ CD8+ T cells. No appreciable populations of CTLA-4-positive T cells were detected in tumors of either model. Together, these data demonstrated interesting immunologic differences between MB subtypes that further corroborated previous observations in human MB samples by Margol et al.

Interestingly, we found that differences in functional immune subsets were associated with differential therapeutic responses to immune checkpoint blockade. Treatment with anti-PD-1 alone or in combination with anti-CTLA-4 conferred a significant survival benefit in Group 3 animals only and not SHH-group animals ($p = 0.02$ and $p = 0.009$, respectively). Neither tumor subtype responded to treatment with CTLA-4 blockade alone. Our findings differ with numerous reports that have identified PD-1 receptor ligand PD-L1 as a significant predictor of response to anti-PD-1 therapy. While we observed significantly higher percentages of PD-1+ CD8+ T cells in Group 3 animals, both tumor subtypes expressed marginal levels of PD-L1 surface expression, with the predominant source stemming from non-tumor cell populations within the tumor microenvironment such as infiltrating myeloid cells. Therefore, PD-L1 expression within these murine models of MB was not a predictor of response to PD-1 blockade. Alternative mechanisms for differential response to immune checkpoint blockade are currently under investigation within our laboratory.

Recent work from Le et al. reported significantly higher immune-related objective responses and progression-free survival in metastatic colorectal patients with mismatch repair deficiencies compared to repair-proficient patients. They demonstrated that tumors with high numbers of somatic mutations due to mismatch-repair deficiency were more responsive to immune checkpoint blockade, likely due to a higher burden of unique immunogenic antigens present within these tumors that can elicit an immunologic response within the host. The mutational landscape of Group 3 tumors has been characterized by significant chromosomal aberrations and epigenetic changes that are also more numerous in comparison to
While we did not detect anti-PD-1 mAb on TILs in symptomatic animals, we cannot rule out that antibody may enter the tumor site at an earlier time point or that antibody was present below the limit of detection. Receptor occupancy studies revealed an absence of anti-PD-1 mAb bound to tumor-infiltrating lymphocytes (TILs) despite a clear presence on peripheral lymphocytes in the spleen and lymph nodes. While we did not detect anti-PD-1 mAb on TILs in symptomatic animals, we cannot rule out that antibody may enter the tumor site at an earlier time point or that antibody was present below the limit of detection.

Figure 1. Anti-PD-1 therapy in Group 3 MB tumors leads to tumor regression and increases survival. (A) Under normal conditions, the tumor microenvironment is populated with immunosuppressive tumor infiltrating macrophages, including TAMs and MDSCs that express PD-L1. T cells positive for PD-1 are also abundant in the tumor microenvironment. Group 3 tumors express marginal levels of surface PD-L1 (not shown). (B) Treatment with PD-1 blockade in Group 3 animals conferred a significant survival benefit over tumor-only controls. Analyses of the tumor microenvironment showed an increase in CD3+ PD-1+ infiltrating T cells. Receptor occupancy studies revealed an absence of anti-PD-1 mAb bound to tumor-infiltrating lymphocytes (TILs) despite a clear presence on peripheral lymphocytes in the spleen and lymph nodes. While we did not detect anti-PD-1 mAb on TILs in symptomatic animals, we cannot rule out that antibody may enter the tumor site at an earlier time point or that antibody was present below the limit of detection.

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SHH-group tumors. Collectively, these findings suggest that the differential immune microenvironments and potentially altered genomic landscapes among subtypes of MB can be leveraged to immunologically target tumors by blocking key immunoregulatory pathways that promote tumor progression.

Our studies additionally suggested a systemic mechanism by which PD-1 blockade induces immunity in the brain. Analysis of PD-1 receptor occupancy revealed an absence of anti-PD-1 mAb on tumor-infiltrating lymphocytes (TILs), despite being bound to T cells within peripheral lymphoid organs including the spleen and tumor-draining cervical lymph nodes. While we did not detect PD-1 bound antibody on T cells within the tumor bed, we observed significant influxes of CD3+ T cells that were negative for PD-1 expression within the brain after systemic PD-1 antibody administration. Further phenotyping of the CD3+ tumor infiltrating cells of treated animals revealed CD8+ T cells to be significantly increased in both tumor subtypes and CD4+ T cells to be increased in Group 3 animals only (Fig. 1). The specific characterization of this infiltrating population of PD-1 negative T cells is ongoing. Of particular potential significance, however, is the notion that immune checkpoint inhibitors may not need to reach the tumor microenvironment to mediate antitumor efficacy. This is of particular importance with regard to treating infiltrative CNS tumor cells sequestered behind the blood-brain barrier. While we cannot rule out from our studies that small quantities of antibodies below the threshold of detection may have penetrated the tumor microenvironment and mediated the expansion of tumor-reactive T cells, the lack of detection of PD-1+ TILs with PD-1 antibody bound to their surface at time points where peripheral T cells show robust surface binding suggests an immune activation mechanism that occurs in the periphery and leads downstream to activated T cells capable of persisting in the tumor microenvironment. Ongoing studies are exploring the kinetics and sites of T cell activation during PD-1 blockade within these intracranial tumor models.

In summary, we demonstrate that the relationship between host immunologic surveillance and the molecular subtype of MB tumors is complex, yet closely intertwined. Our recent work published in Clinical Cancer Research showed that among distinct immune-phenotypes, anti-PD-1 therapy conferred superior antitumor activity in Group 3 tumor-bearing animals, but not in the SHH-group animals. Although less responsive to immune checkpoint inhibitors, it is possible that selective blockade of a different immunosuppressive subset, such as TAMs or MDSCs, may be required to achieve similar antitumor efficacy in the SHH group. As the title of this review suggests, understanding how the cellular players within the tumor microenvironment interact in each tumor subtype are likely to unlock potential targets that will have significant implications in the clinical development of immunotherapy targeting MB.

Disclosure of potential conflicts of interest
No potential conflicts of interest were disclosed.

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