Glioblastoma multiforme (GBM) is the most common primary brain tumor in adults and has a very poor prognosis. Part of the challenge in treating GBM is related to its localization within the naturally immunosuppressive central nervous system (CNS), which is anatomically-protected by a blood brain barrier and devoid of the traditional lymphatic structure. Interestingly, even with these specialized immunological restrictions, leukocytes are known to traverse the CNS and invade GBMs.2

Of the various T cell subsets, regulatory T cells (Treg; CD3+CD4+Foxp3+CD25+) are selectively enriched in GBM-infiltrating CD4+ T cell pool3 and their neutralization and/or depletion has been correlated with an increase in overall survival.4,5 Given the potently immunosuppressive effector function of Treg, therapeutic strategies that diminish their suppressive effect are an area of active research.6,7 The Treg levels reported in GBM suggest that this cell type could be a major contributor to the immunosuppressive microenvironment within this tumor.8

Notably, we found that the triple immunotherapeutic approach was ineffective in generating long-term survival in mice with intracranial B16/F10 melanoma-based tumors. While this likely reflects the overall aggressiveness of melanoma, it may also reflect a selective permissiveness that melanoma cells gain after accessing the CNS. We demonstrated that there was a significant decrease in Treg levels between intracranial GL261 and B16/F10 tumors [27 ± 7% and 2 ± 0.3% Treg (P < 0.01), respectively] when analyzed at 12 d post-injection. Although the mechanism causing Treg recruitment was not explored in our study, it is interesting to hypothesize that the triple immunotherapeutic approach is more effective against intracranial tumors that utilize Tregs as a dominant immunosuppressive mechanism. Future studies will be necessary to further elucidate this point.

As the title of this review suggests, more may mean better when it comes to combinations of immunotherapeutic approaches. However, the efficacy of this triple approach may be highly context-dependent, as illustrated by the marked discrepancy between the survival and therapeutic effect of the CTLA-4/PD-L1/IDO blockade utilizing CTLA-4, PD-L1 mAb and/or IDO inhibition in patients with GBM is worthy of a phase 1 clinical trial.9

Collectively, these pre-clinical data provide promising evidence that combination therapy utilizing CTLA-4, PD-(L)1, and/or IDO inhibition in patients with melanoma-based tumors. While this likely reflects the overall aggressiveness of melanoma, it may also reflect a selective permissiveness that melanoma cells gain after accessing the CNS. We demonstrated that there was a significant decrease in Treg levels between intracranial GL261 and B16/F10 tumors [27 ± 7% and 2 ± 0.3% Treg (P < 0.01), respectively] when analyzed at 12 d post-injection. Although the mechanism causing Treg recruitment was not explored in our study, it is interesting to hypothesize that the triple immunotherapeutic approach is more effective against intracranial tumors that utilize Tregs as a dominant immunosuppressive mechanism. Future studies will be necessary to further elucidate this point.

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modulating the immune response against tumors. This concept may be somewhat problematic from the standpoint of clinical trial development, since the majority of early phase trials focus on evaluating the safety and potential efficacy of single agents. However, such an approach, in the opinion of the authors, represents a missed opportunity since it assumes that cancer immunotherapy can be achieved via modulation of a single pathway. In fact, as we propose here and is shown in our published work in the setting.

In summary, our work provides a proof-of-concept for reversing glioma-induced immunosuppression via the incorporation of multiple partners in the clinical setting. As shown in our published work in Clinical Cancer Research, simultaneously administered triple therapy of mice with established brain tumors led to 100% overall survival. Whether we can achieve similar results in patients remains to be seen. However, the design and execution of a clinical trial focused on CTLA-4/PD-L1/IDO inhibition in malignant glioma represents a most reasonable and timely approach, given the availability of all three reagents for use in the clinical setting.

Disclosure of Potential Conflicts of Interest

The authors declare that no competing interests exist.

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Figure 1. Simultaneously targeting CTLA-4, PD-L1 and IDO decreases Treg and increases survival. (A) Under normal conditions, primary brain tumors express IDO, a cytoplasmic enzyme that catabolizes the essential amino acid, tryptophan (Tryp.), to the downstream metabolite, kynurenine (Kyn). Based on recent studies showing that kynurenine interacts with the aryl hydrocarbon receptor in CD4+ T cells to increase FoxP3 expression, this may be a primary mechanism contributing to Treg expansion in the brain tumor. Coincidently, CTLA-4 is constitutively and highly expressed on the tumor-resident Treg population that can both directly inhibiting conventional CD4+ T (Tconv) function, as well as interact with surrounding macrophages, microglia and/or dendritic cells (not shown) via B7.1 and B7.2 to induce IDO expression. Finally, PD-L1 is highly expressed by both tumor cells, as well as tumor-infiltrating macrophages, both of which contribute to the highly immunosuppressive tumor microenvironment. When CTLA-4 and PD-L1 mAbs, in addition to the IDO chemical inhibitor, 1-methyl tryptophan (1-MT), is combinatorially administered to mice bearing brain tumors, (B) Treg levels decline, presumably by the decreased kynurenine availability, contributing to the Tconv-mediated assistance to CD8+ cytotoxic T lymphocytes (CTL), which produce higher interferon-gamma levels (not shown) that contribute to brain tumor clearance and increased survival. It should be noted that, although the PD-L1 and CTLA-4 mAbs, as well as 1-MT, are schematically represented here to be acting directly within the tumor microenvironment, we cannot rule out that their primary mechanism of action is limited to the central nervous system compartment. Cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed cell death ligand 1 (PD-L1) and indoleamine 2,3-dioxygenase (IDO)
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