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Is there a role for neoadjuvant anti-PD-1 therapies in glioma?

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Abstract

Purpose of Review: In this review, we summarize recent findings that highlight the progress for checkpoint blockade immunotherapy in glioblastoma patients.

Recent Findings: We review new data from our group and others that suggest that the timing of when immunotherapy is applied can impact the anti-tumor immune response and, potentially, the ultimate clinical benefit of patients.

Summary: The neoadjuvant priming and expansion of exhausted T cells within the GBM microenvironment, followed by the removal of an immune suppressive tumor microenvironment through surgical resection, may lead to enhanced anti-tumor immune responses that beneficial clinically. As such, neoadjuvant immunotherapeutic approaches and rational combinations may be helpful scientifically to understand how immunotherapeutic interventions influence the tumor microenvironment, as well benefit the patients.

Keywords

Checkpoint blockade; Neoadjuvant immunotherapy; Glioblastoma

Introduction

As checkpoint blockade immunotherapy assumes an increasingly central role in cancer treatment, continued efforts to exploit anti-PD-1 monoclonal antibody blockade for the treatment of patients with glioblastoma (GBM) remain unsuccessful. As of yet, there are still no FDA-approved brain tumor checkpoint blockade immunotherapies. Many challenges contribute to this lack of progress, some that we understand and many that we do not.

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3.Conflicts of Interest

None

Although it has become clear that the central nervous system (CNS) is not as hermetically immunoprivileged and immunologically inaccessible as previously thought (1), the CNS is nevertheless immunologically distinctive such that the basic mechanisms of immune system activation present in extracranial compartments cannot be easily extrapolated to the brain. Specifically, there are major gaps in our understanding of fundamental components of the immune response to brain tumors, such as the identification of critical antigen presenting cells, the location of naïve T cell priming, and the details underlying effector T cell trafficking into brain tumors, among others. Consequently, therapies utilizing checkpoint blockade in an adjuvant setting have seen success in many cancer types (2), however the efforts directed at understanding its efficacy in GBM remain unsuccessful. Anti-PD-1(Nivolumab) immunotherapy administered did not improve survival in unselected recurrent GBM patients, and recent studies of Nivolumab in newly diagnosed patients failed to reveal significant clinical responses(3, 4). Thus, the biological features specific to GBM and anatomic features specific to the brain contribute to the difficulties of identifying and implementing effective brain tumor checkpoint blockade immunotherapy.

Despite the challenges to brain tumor immunotherapy development, recent work in the field points to neoadjuvant anti-PD-1 checkpoint blockade as a promising and necessary progression of treatment for GBM. In this review, we will discuss the current limitations of anti-PD-1 therapy before discussing the recent clinical trials and Next-Generation Sequencing analysis on the pre-surgical, neoadjuvant administration of anti-PD-1 immunotherapy for GBM.

Current Limitations to anti-PD-1 therapy in GBM

Due to the success of checkpoint blockade immunotherapy (ICI) in many cancer types (5–8), significant work has been directed in establishing this treatment for GBM (9). To date, these efforts have been unsuccessful. Anti-PD-1 immunotherapy failed to improve survival or reveal significant clinical responses in recurrent GBM patients (3, 4, 10). Multiple factors could underlie these disappointing clinical outcomes. The first one is associated with the immune privilege of the brain, where brain tumors demonstrate a ‘cold tumor phenotype’ with very few tumor-infiltrating lymphocytes and other immune effectors (11, 12). Another factor that caused immunosuppression or-evasion is the significant immunoediting during GBM tumor development (13), due to its high genomic heterogeneity (14) or epigenetic plasticity (15). Many studies have described severe immunologic impairments observed in GBM, including telomere-induced T cell senescence anergy, exhaustion, ignorance, and immune tolerance (16). Although specific biomarkers (such as PD-L1 expression, tumor mutational burden, etc) are associated with responses to ICI in several cancer types (17, 18), to date, we have little understanding of the intrinsic properties that render some gliomas susceptible or resistant to immunotherapy. Therefore, clinical trials designed under the premise that all tumors are equally susceptible may not be able to demonstrate efficacy. Additional complementary studies have begun to explore a more fundamental question in the use of checkpoint blockade in GBM—i.e., the timing of the treatment. Thus, identifying *who* may benefit from checkpoint blockade and *when* checkpoint immunotherapy should be administered represent new ways to think about the utility of these therapies in treating GBM.

Checkpoint blockade has traditionally been administered to GBM patients in the adjuvant setting, mirroring the use of temozolomide (19). However, recent pre-clinical and clinical studies implicated that neoadjuvant administration may stimulate a more robust anti-tumor immune response and lead to objective clinical response and survival in various solid tumors. For example, in a recent comparison study in stage III melanoma, two doses of concurrent ipilimumab (3 mg/kg) and nivolumab (1 mg/kg) prior to surgery resulted in a complete pathologic response at surgery in 7 of 9 patients (20). In a follow-up trial, where the dose strategy was modified, the inverstigators again reported a high response rate (77%) to neadjuvant combination ICI with a lower toxicity level (21). In another recent study, neoadjuvant PD-1 blockade induced a 45% pathological response in patients with non-small cell lung cancer (22). Notably, after PD-1 blockade, T cell clones identified within tumors and blood were shown to expand in a subset of patients. More importantly, multiple studies have also shown the clinical benefit of neoadjuvant ICI in patients with melanoma brain metastases, which may be subjected to the same environmental cues as GBM within the central nervous system (CNS) (23, 24).

Neoadjuvant Immunotherapy Clinical Trials for GBM

As we begin to understand more about identifiable biomarkers that correlate with response and resistance to checkpoint blockade, recent studies have started to examine whether these drugs are “on target” and truly stimulate observable immune responses in the GBM microenvironment. Conceptually, in order to undergo a clinically relevant response to agents blocking the PD-1/PD-L1 molecules, the host must have first developed an antitumor response, likely antigen specific, which was prevented from effectively attacking tumor cells due to the engagement of the PD-1/PD-L1 pathway. However, unless the unique features of the tissue-specific GBM microenvironment are characterized in patients following checkpoint blockade, it is difficult to determine the effects of these treatments on the tumor-immune system dynamic and to understand the molecular basis for intrinsic resistance. To this end, “window of opportunity” clinical studies are well suited to enable comprehensive interrogation of the immune response in GBM following treatment (13). In this method, patients are treated neoadjuvantly prior to surgery or biopsy.

This approach has recently been extended to recurrent GBM with provocative data from three independent studies underscoring the need for further study. In Cloughesy et al, patients were enrolled in a randomized clinical trial patients designed to compare neoadjuvant plus adjuvant PD-1 blockade with adjuvant PD-1 blockade only(25). One dose of pembrolizumab (200 mg) was administered prior to surgical resection in the neoadjuvant group, and both groups were treated with the same dosing every 3 weeks following resection until progression. In responding patients, a transcriptional signature highlighted by high expression of T cell and interferon genes, but downregulation of cell cycle-related genes, was differentially expressed in tumor tissue of patients who received neoadjuvant pembrolizumab. Moreover, an expansion of T cell clones in peripheral blood and induction of PD-L1 in the tumor tissue was observed. These data were concordant with the study by Schalper and Melero (26), who also observed the induction of an interferon signature in the tumor tissue and changes in T cell receptor clonality. Schalper et. al. confirmed PD-1 receptor occupancy by nivolumab in brain tumor tissue, addressing a longstanding

question that the blood brain barrier may not limit the re-invigoration of anti-tumor immune responses *in situ*. Together with the report of Zhao et al (27), these studies showed similar immunologic findings that, in patients with recurrent GBM, PD-1 blockade could induce T cell activation and infiltration into the GBM tumor microenvironment. However, when these data are considered along with the other adjuvant trials in glioblastoma, we believe that the limited therapeutic effect of neoadjuvant PD-1 blockade is likely due to (1) the insufficient number of T cells entering the tumor parenchyma and (2) microenvironmental cues that impeded the tumor antigen-specific T cells from becoming fully functional effectors.

The mechanisms preventing a clinical response in recurrent GBM are currently unknown but are likely multifactorial. Notably, patients who were randomized to receive neoadjuvant pembrolizumab in the Cloughesy et al study lived, on average, twice as long as the patients who received only adjuvant pembrolizumab (25), although no survival benefit compared to historical controls was observed among neoadjuvant nivolumab recipients in the Schalper study(26). Although this finding might be simply related to chance, another plausible hypothesis is that the neoadjuvant priming and expansion of exhausted T cells within the GBM microenvironment, followed by the removal of an immune suppressive tumor microenvironment through surgical resection, led to the observed prolonged survival. A mechanistic underpinning for these data is not yet fully understood. However, pre-clinical studies and clinical immune monitoring work have shown that neoadjuvant immunotherapy was associated with the rapid expansion of tumor-specific $CD8^+$ T cells in other solid tumors(20, 28). Some of these expanded $CD8^+$ T cell clones found in the systemic circulation could be identified in the primary tumor, while other expanded tumor-specific T cell populations seem to be clonally replaced following PD-1 blockade (22, 29, 30). Crucially, the presence of the primary tumor prior to extirpation appears to be critical for the efficacy of the neoadjuvantly administered immunotherapy, suggesting that factors such as (1) the absolute amount of antigen burden during immune cell licensing as well as (2) the presence of tumor-involved antigen presenting cells are critically involved in the reinvigoration of antigen-specific T cells. In mouse models, cross presentation by $Batf3^+$ or $CD103^+$ dendritic cells are involved in effector T cell trafficking into the tumor microenvironment (31). Thus, available data suggest that a complex dynamic of T cell reinvigoration, chemokine cues for trafficking, and available antigen presentation capacity regulates the infiltration and function of effective anti-tumor immune responses induced by checkpoint blockade.

In the study of Cloughesy et al (25), a randomization strategy was employed into the neoadjuvant component of the clinical trial design that ultimately provided two distinct advantages. First, this approach enabled the use of a contemporary randomized control group prior to surgery, with the same inclusion and exclusion criteria from which to make comparisons of tissue and systemic immune responses against the experimental arm. Randomization is particularly important for these studies and provides additional confidence that observed immunologic and transcriptional findings were robust. Second, randomization facilitated reduced bias in evaluation when comparing arms for a clinical efficacy endpoint. Despite the underpowering of the clinical efficacy endpoints, large effect sizes can be statistically evaluated in order to provide further insight into clinical development opportunities. Randomization will likely be incorporated into the neoadjuvant

element of future studies, and the appropriate trial design of such studies will ideally allow for meaningful comparisons across datasets. Moving forward, additional trials should be initiated in the neoadjuvant setting to better understand how particular drug combinations alter the immune cellular composition and activation states with the tumor microenvironment. Importantly, harmonized assays needed decode the immune responses may allow for meaningful and effective comparisons between trials. As it stands, the current data suggest that there may be opportunities to tune the immunogenicity of GBM if the timing of treatment is “moved up” to the pre-surgical setting. Rigorous studies of these approaches may help to deconstruct the key features of GBM immunoediting currently limiting immunotherapeutic approaches.

Single Cell RNA Sequencing analysis of immune landscape of neo-adjuvant anti-PD-1 therapy.

A recent follow-up study of the Cloughesy et al trial have sought to answer the question: how does neo-adjuvant anti-PD-1 therapy impact the tumor microenvironment and immune landscape? This high-dimensional single-cell study supported the findings from prior neo-adjuvant anti-PD-1 trials (25, 26). More importantly, it provided much deeper insights of how neo-adjuvant anti-PD-1 remodels the cellular immune compositions of the GBM tumor microenvironment (Table 1). Single cell RNA sequencing analysis of patient GBM after neo-adjuvant anti-PD-1 therapy shows increase in the overall T cell infiltrate in the GBM microenvironment and in the proportion of both activated and exhausted T cells with IFN-y activation and cytolytic markers. Further, neoadjuvant anti-PD-1 therapy induced populations of effector T cells to upregulate the secreton of chemotactic factors associated with dendritic cell recruitment, however this was tempered by increases in macrophage immunosuppressive activity (Unpublished data). The immunosuppression associated with the immune microenvironment is compounded by other immunosuppressive mutations in the tumor itself including a significant enrichment of PTEN mutations associated with immunosuppressive expression signatures in patients who did not respond to anti-PD-1 therapy (27). In a similar window of opportunity phase II clinical trial of Pembrozulimab for GBM, CyTOF immune analysis showed low infiltration of CD4+ and CD8+ T cells and high infiltration of CD68+ macrophages into the tumor (32). While the macrophage mediated immunosuppression remains similar, the increased T cell infiltration in the neoadjuvant Pembrozulimab treatment compared to adjuvant administration shows promise for neoadjuvant anti-PD-1 therapy for GBM.

This sequencing data suggests a proposed model of neoadjuvant anti-PD-1 therapy in GBM that first activates T cells in the periphery that migrate into the GBM tumor microenvironment (Fig 1). These activated T cells produce cytotoxic granules that induce tumor apoptosis and chemotactic factors that activate and signal dendritic cells into the tumor. This promotes additional DC and T cell trafficking to the tumor, resulting in cross presentation and IFN-y release that recruits additional immune cells. Some of these tumor antigen-specific T cells transition to a progenitor exhausted phenotype while the IFN-y stimulates immunosuppressive molecules, ultimately attenuating the response of neoadjuvant anti-PD-1 therapy (Unpublished data). Although neoadjuvant anti-PD-1

therapy improves survival outcomes, as of yet, it is clear that such therapy is not completely curative as positive T cell responses are mitigated by neoadjuvant anti-PD-1 induced immunosuppressive ones. Continuing studies into the mechanism of various immunosuppressive factors like macrophages and other myeloid-derived suppressor cells may establish combinatorial therapy strategies like dual-ligand neoadjuvant anti-PD-1 and anti CTLA-4 or immune checkpoint blockade and dendritic cell vaccination that will increase the efficacy of neoadjuvant anti-PD-1 and further boost the anti-tumor T cell immunity in GBM (33, 34).

Conclusion

We have summarized exciting recent progress in neoadjuvant anti-PD-1 checkpoint blockade immunotherapy for GBM. Rational clinical trial design and prudent patient stratification remain critical to these efforts in order to not only identify those subsets of patients most likely to benefit from new treatments but also to avoid ignoring a clinical signal within the noise of a heterogeneous treated population. To this end, it is likely that next generation sequencing approaches will remain particularly important in order to understand the genomic basis of response and resistance to checkpoint blockade therapy. Combining these strategies rationally will likely prove pivotal in bringing more effective treatments to patients. Ultimately, as we study these exciting approaches in larger studies, we will learn not just about their therapeutic efficacy of neoadjuvant anti-PD-1 therapy but also gain new insights into the fundamental immunobiology of the CNS itself.

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Key Points

- Neoadjuvant or Window of Opportunity trials provide a rational clinical trial design to investigate how immunotherapy alters the tumor microenvironment of glioblastomas.
- Neoadjuvant checkpoint blockade immunotherapy may induce an anti-tumor T cell immune response that is enhanced followed the planned surgical resection of the tumor.
- A more in-depth understanding of the cellular interactions within the tumor microenvironment will allow for rational combinations that may induce more effective anti-tumor immune responses and, potentially, clinical responses.

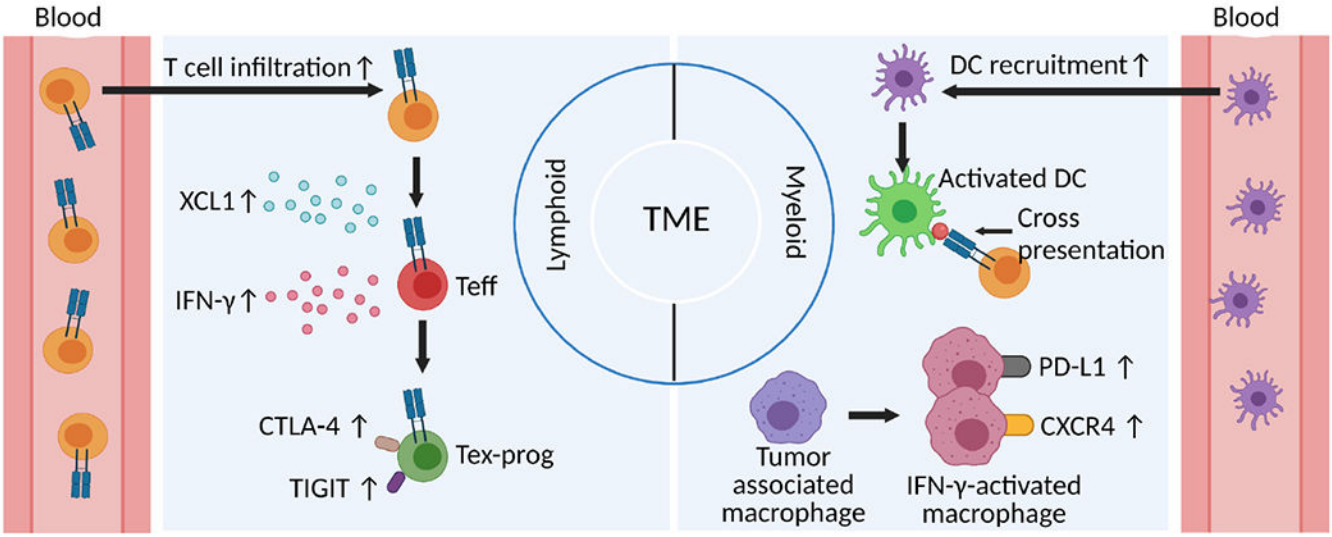

Figure 1.

Proposed model of immune remodeling in GBM elicited by neo-adjuvant anti-PD-1. TME, tumor microenvironment

Table 1

The summary of major findings associated with neo-adjuvant anti-PD-1 treatment in GBM

Study	Cancer type		Technique					Effects of neoadjuvant anti-PD-1
	Recurrent GBM	Newly diagnosed GBM	Bulk mRNA analysis	TCRseq	IF	CyTOF	scRNAseq	
Cloughesy et al.	√		√	√	√	√		Upregulation of T cell- and interferon-y-related gene expression
								Downregulation of cell-cycle related gene expression
								Focal induction of PD-L1 in the tumor microenvironment
								Upregulation of immune checkpoints (e.g., CTLA-4, CD276 and LAG3)
Schalper et al.	√	√	√	√	√			Higher proportion of tumor-infiltrating immune cells
								Upregulation of chemokine transcripts (CXCL10, CCL4, and CCL3L1)
								Increased TCR clonal diversity of tumour infiltrating T cells
Unpublished single cell omics study	√	√			√	√	√	Increased tumor infiltrating T cells and higher proportion of progenitor exhausted T cells
								Enhanced interferon-y production in the tumor microenvironment
								Activated T cells produced chemokines (XCL1, XCL2) that recruit dendritic cells
								Upregulation of T cell checkpoints CTLA4 and TIGIT
								Induction of CXCR4+ and PDL1+ tumor-associated macrophage populations

IF, immunofluorescence