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TOPIC REVIEW

Glucocorticoids and immune checkpoint inhibitors in glioblastoma

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Abstract

Purpose Immunotherapy, activation of the immune system to target tumor cells, represents a paradigm shift in the treatment of cancer. Immune checkpoint therapies, which target immunomodulatory molecules expressed on T-lymphocytes, have demonstrated improved survival in a variety of malignancies. However, benefit in glioblastoma, the most common and devastating malignant brain tumor, remains to be seen. With several recent clinical trials failing to show efficacy of immunotherapy, concerns have been raised regarding the impact of glucocorticoid use in this patient population that may impair the ability for immune checkpoint inhibitors to affect a response.

Methods For this article we examined the mechanism by which immune checkpoint inhibitors activate, and glucocorticoids impair, T-lymphocyte function.

Results In this context, we review the clinical data of immune checkpoint inhibitors in glioblastoma as well as the impact glucocorticoids have on immune checkpoint inhibitor efficacy. Finally, we highlight key questions that remain in the field, and the potential benefit of further research for central nervous system tumors.

Conclusion More information on the extent, character and duration of glucocorticoids on patients treated with PD-(L)1 will better inform both clinical management and novel therapeutic development of immunotherapy in patients with CNS malignancies.

Keywords Glucocorticoids · Steroids · Immune · Checkpoint · CNS · Glioblastoma

Introduction

Despite much effort over the past several decades, glioblastoma, the most common malignant brain tumor, remains a disease with dismal prognosis, debilitating comorbidities and limited therapeutic options. Immune checkpoint inhibitors offer the potential for highly-tolerable therapy with durable responses across a variety of cancer types. Much interest has rightly arisen for employing these treatments in patients with glioblastoma. However, to date phase II and III studies have been disappointing. A leading concern for this apparent lack of efficacy is the high prevalence of glucocorticoid use among glioblastoma populations, a necessary consequence of cerebral edema and radiotherapy treatments. An improved understanding of the interplay between immune checkpoint inhibitors, CNS tumors and glucocorticoids may therefore offer the key to more effective trial design and improved clinical management going forward.

Immune checkpoint blockade

Immune checkpoint inhibitors have assumed a preeminent role in the treatment of many solid tumor and hematological malignancies, including non-small cell lung cancer (NSCLC), renal cell cancer (RCC), melanoma, breast cancer and Hodgkin's lymphoma [1–5]. By modulation of cellular elements within the tumor microenvironment (TME), an anti-tumoral, pro-inflammatory response can be achieved. In the TME, cytotoxic T cells expressing the co-receptor CD8 recognize tumor peptides on antigen presenting cells (APC). This recognition is accomplished through the interaction of the CD3 T Cell Receptor (TCR) with the Major Histocompatibility Complex (MHC) but modulated by multiple costimulatory (also known as second signal) and inhibitory mechanisms. Programmed Death Ligand (PD-L1), an

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inhibitory immunoglobulin expressed on tumors, deactivates T cells via binding its cognate ligand PD-1 [6, 7]. Similarly, the inhibitory receptor Cytotoxic T Lymphocyte protein 4 (CTLA-4) is expressed by T cells and competes with the costimulatory molecule CD28 to bind CD80/86 [8, 9]. When CTLA-4 is engaged with CD80/86, CD28 is unable to bind and transmit an activation signal [10]. By blocking these PD-L1 or CTLA4 interactions, immune checkpoint inhibitors incite T lymphocytes towards proliferation, differentiation and ultimately targeting of the malignancy.

Immune checkpoint inhibitors in CNS tumors

Initial results from immune checkpoint inhibition in glioblastoma have been disappointing. Recently published results from phase I exploratory cohort of CheckMate 143 describe 40 patients with recurrent glioblastoma, treated with nivolumab, a PD-1 inhibitor, alone or in combination with the CTLA-4 inhibitor ipilimumab [11]. 68% of these patients expressed PD-L1 ($\geq 1\%$). Overall survival (OS) was 10.4 months in those receiving nivolumab 3 mg/kg, 9.2 months with nivolumab 1 mg/kg + ipilimumab 3 mg/kg, and 7.3 months after nivolumab 3 mg/kg + ipilimumab 1 mg/kg. Combination therapy was poorly tolerated with 20–30% of patients experiencing adverse events which lead to discontinuation. A phase III cohort of CheckMate 143 reported that nivolumab monotherapy does not improve survival as compared with bevacizumab, a VEGF inhibitor approved for second line use [12].

Similarly, phase III data is pending on the efficacy of checkpoint blockade in the newly-diagnosed population but early reports indicate that PD-1 inhibition does not drastically improve outcomes in an unselected population. CheckMate-548, a randomized, multicenter trial of patients with newly-diagnosed MGMT-methylated glioblastoma treated with temozolomide and radiation, showed that the addition of nivolumab did not show a statistically significant improvement of progression-free survival (PFS). The study remains open to allow for OS data to mature [13]. Similarly, CheckMate-498 examined newly-diagnosed MGMT-unmethylated patients treated with radiation (without temozolomide), and randomized to either concurrent and maintenance nivolumab or no additional therapy [14]. The study did not meet its primary endpoint of OS with publication of the full evaluation and subsequent results forthcoming soon.

With these findings in mind, a recent study from the Ivy Foundation Consortium has cast renewed focus on immune checkpoint modulation in glioblastoma, specifically in the perioperative setting. This randomized, open-label pilot study compared neoadjuvant to adjuvant pembrolizumab, a PD-1 inhibitor, in 35 patients with recurrent, surgically resectable glioblastoma. Patients receiving pembrolizumab prior to surgical resection had significantly improved OS (13.7 months vs 7.5 with adjuvant pembrolizumab) [15]. Although PFS was also improved (3.3 months vs 2.4 respectively) the difference between arms was much less than that observed in OS indicating that the potential mechanism for improved survival is not directly mediated by reduced tumor volume. Transcriptomic analysis identified increased interferon- and T cell-pathway signaling in the neoadjuvant as compared with the adjuvant arm. Immunofluorescent examination showed that neoadjuvant specimens were more likely to have focal PD-L1 expression with a high CD8 infiltration. TCR sequencing of peripheral blood mononuclear cells showed that the patients receiving neoadjuvant pembrolizumab had expanded clonality rearrangements. Similarly, a phase II trial of 40 patients with glioblastoma (27 recurrent, 3 newly diagnosed) treated with neoadjuvant nivolumab, followed by surgery and adjuvant nivolumab had upregulation of chemokine transcripts, increased immune cell infiltration and TCR clonal diversity among tumor-infiltrating lymphocytes (TILs) [16]. Finally, a molecular analysis of 66 patients with glioblastoma treated with PD-1 inhibitors highlighted the role of MAPK pathway alterations, such as BRAF in patients who respond to immunotherapy [17]. In this study patients were classified as responders if tissue samples showed immune infiltration with few to no tumor cells or if tumor volumes were radiographically stable or shrinking over at least 6 months. MAPK inhibitors have shown synergism with PD-1 blockade in murine models [18]. Additionally, the study found that PTEN mutations were enriched among non-responders. In melanoma preclinical models, PTEN loss has previously been shown to increase immunosuppressive cytokines and in turn inhibit TIL quantity and function.

Glucocorticoid use in CNS tumors

Glucocorticoids are naturally produced in the adrenal cortex where they play various roles in the homeostatic regulation of inflammation, metabolism, sodium regulation and the immune response. Upon binding glucocorticoid receptor in the intracellular space, the receptor dissociates from its inhibitory proteins, dimerizes and then translocates into the nucleus where it regulates gene transcription. [19]. Glucocorticoids have also been shown to regulate transcription via a more direct mechanism [20]. In addition to the treatment of cerebral edema and numerous other autoimmune conditions, steroids remain a cornerstone for the treatment of radiation necrosis. Glucocorticoids are also the primary therapy for immune-related adverse events (irAE) secondary to immune checkpoint inhibitors. In general corticosteroids

are employed for grade II irAE although this varies according to the specific toxicity involved [21].

Dexamethasone, a synthetic glucocorticoid, is the most commonly used glucocorticoid in patients with CNS malignancies. Dexamethasone is indicated for, and universally employed in, the treatment of cerebral edema, a common occurrence in patients with CNS tumors [22, 23]. As dexamethasone possesses high glucocorticoid and low mineralocorticoid activity it has less impact on the renin-angiotensin-aldosterone system, and in turn sodium retention, then naturally occurring glucocorticoids such as hydrocortisone. Complications of long-term dexamethasone use include the suppression of the hypothalamic-pituitary adrenal axis, infection, cataracts, peptic ulcers, osteoporosis, myopathy and psychosis. It has linear pharmacokinetics with a plasma half-life of 4 hours [24, 25] and reaches a maximal plasma level between 1.6 and 2.0 hours after administration. The relative bioavailability of oral dexamethasone is 70–81% compared to intramuscular administration [26] with an AUC of approximately 774ug. Twice daily dosing provides an appropriate clinical response [27].

There is increasing evidence that glucocorticoid exposure is an independent predictor of survival in glioma, and that this effect is mediated by immunosuppression at the level of the tumor micro-environment. Randomized trials in murine glioblastoma models have clearly demonstrated that corticosteroid pretreatment prior to irradiation decreases-survival [28]. The impact of corticosteroids on human subjects however, is less clear. Arguments for this detrimental effect include a study of 832 patients with glioblastoma which found that corticosteroid-administration at the time of radiotherapy initiation was an independent predicator of decreased survival even after adjusting for resection extent, initial treatment, age and Karnofsky Performance Score (KPS). Likewise, a post-hoc analysis of patients receiving tumor-treating fields found that dexamethasone doses greater than 4.1 mg daily had decreased OS (4.8 months vs 11.0 in those receiving $\leq$4.1 mg daily) [29]. This segregation of OS among groups persisted even after accounting for KPS, age and tumor size. Absolute CD3 T-lymphocyte count was found to be the strongest predictor of survival (2.0 months with $\leq$382cells per mm^3 vs 7.6 months with > 382 cells per mm^3) but CD4 and CD8 subsets also correlated with survival. However, these results are in contrast to a prior study of 76 patients with high grade glioma treated with corticosteroids which found that CD4 counts < 200 mm^3 (occurring in 24% of patients) did not impact survival [30]. Further evidence that glucocorticoid exposure may not directly alter tumor-infiltrating lymphocytes (TIL) density comes from a retrospective immunohistochemical analysis which found no correlation between tumor-infiltrating lymphocytes and preoperative GC in 135 glioblastoma specimens [31]. Likewise, immunohistochemical analysis in 116 brain metastasis specimens also showed no correlation [32]. TIL density had been the primary objective for the IVY Foundation trial comparing neoadjuvant to adjuvant PD-1 blockade. Although variability in the neoadjuvant cohort was appreciated there was not statistical difference in CD8 TILs between neoadjuvant and adjuvant groups. Importantly, work in our laboratory demonstrated that exposure of isolated T lymphocytes to dexamethasone did not cause a significant drop in cell number but prevented the proliferation of naïve T cells when exposed to immune stimulation [33]. These results are in concert with the tumor data where corticosteroids do not decrease the TIL population.

Glucocorticoid inhibition of T lymphocytes

Early work in the 1970s showed that limited glucocorticoid administration could induce lymphopenia within 4–6 h and that this lymphopenia recovered within 24 h [34]. Sequestration of peripheral lymphocytes into the bone marrow was

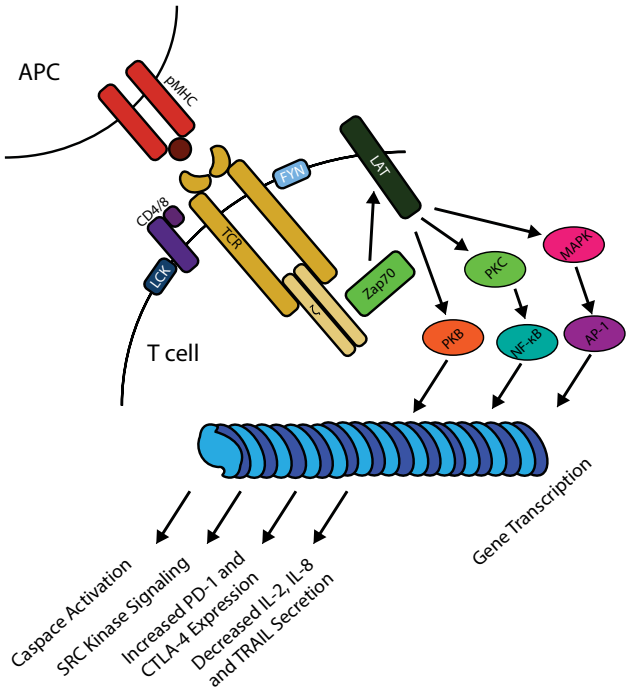

Fig. 1 Effect of Glucocorticoids on T cell Signaling. Activator protein 1 pathway (AP-1), antigen-presenting cell (APC), cluster of differentiation (CD), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), proto-oncogene tyrosine-protein kinase (FYN), interleukin (IL), linker for activation of T cells (LAT), lymphocyte-specific protein tyrosine kinase (LCK), mitogen-activated protein kinase pathway (MAPK), nuclear factor kappa B pathway (NF-κB), peptide major histocompatibility complex (pMHC), programmed cell death protein 1 (PD-1), protein kinase B pathway (PKB), protein kinase C pathway (PKB), T cell receptor (TCR), TNF-related apoptosis-inducing ligand (TRAIL), zeta-chain (ζ), zeta-chain-associated protein kinase 70 (ZAP70)

implicated as a potential mechanism for this rapid shift and later studies using fluoresceinated peripheral blood lymphocytes demonstrated that diminished efflux from lymphoid organs also played a role, albeit a diminished one if glucocorticoid exposure is prolonged [35, 36].

Several molecular pathways have been described regarding the immunosuppressive effects of glucocorticoids on T lymphocytes (Fig. 1). Dexamethasone inhibits TCR signaling via interruption of membrane-proximal phosphorylation events as evidenced by decreased phosphorylation of zeta chain, ZAP70 kinase and the adaptor molecule linker of activation of T cells (LAT) [37]. Furthermore, glucocorticoids similarly decrease phosphorylation of Lck and Fyn, mediators of the TCR-CD4 and CD3 interactions respectively. This results in downregulation of protein kinase B (PKB), protein kinase C (PKC) and mitogen-activated protein kinase (MAPK) pathways [38]. Importantly, this TCR inhibition appears to be through a glucocorticoid receptor dependent mechanism. This inhibition of TCR signaling in turn leads to apoptosis via steroid receptor coactivator (SRC) kinase signaling and caspase activation [39]. Glucocorticoids have been shown to negatively regulate interleukin 2 (IL-2) transcription. Additionally, glucocorticoids were shown to downregulate IL-2 production by acting upon the IL-2 promoter and its transcription factor AP-1, although it should be noted that this effect may also be concentration dependent [40, 41]. There have been contradictory reports on the ability for exogenous IL-2 to rescue T cells from glucocorticoid-induced inhibition [42, 43].

Much is known about the modulation of hematopoietic cells by glucocorticoids (Fig. 2). Among CD4 + T cells, naïve cells were noted to have increased sensitivity to dexamethasone inhibition compared to memory T cells, and CD28 and PKC mechanisms were implicated as potentially responsible for this difference. Comprehensive phenotyping of 20 healthy volunteers treated with a single dose of hydrocortisone confirms that naïve T cells account of the predominance of CD4 + T cell subpopulation loss [44]. In contrast, effector memory, helper memory and Th17 cell frequencies were increased after glucocorticoid exposure. Similarly, among CD8 + T cells, naïve T cells were decreased while

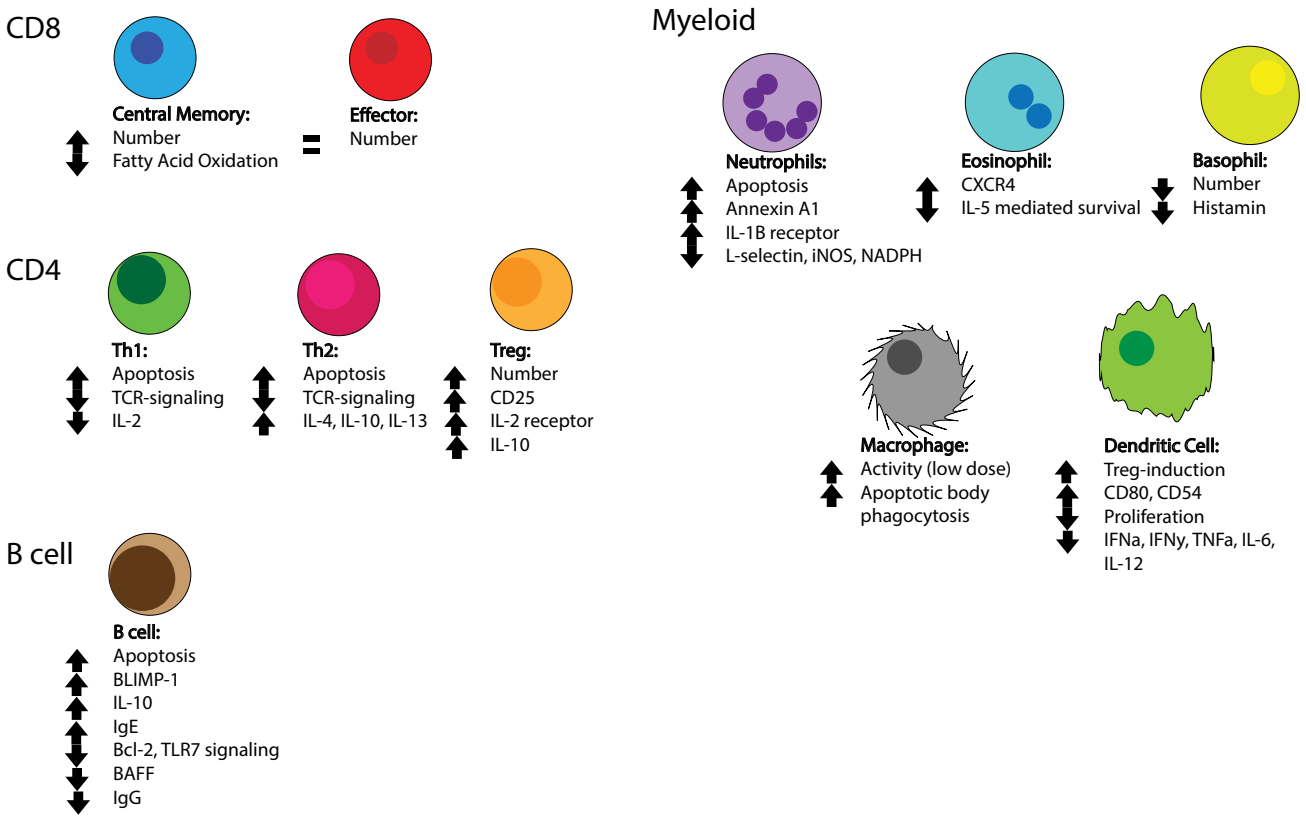

Fig. 2 Effect of Glucocorticoids on Hematopoietic Cells. B cell activating factor (BAFF), B cell lymphoma 2 (BCL-2), B-lymphocyte-induced maturation protein 1 (BLIMP-1), cluster of differentiation (CD), C-X-C chemokine receptor 4 (CXCR4), immunoglobulin (Ig), inducible nitric oxide synthase (iNOS), interleukin (IL), interferon alpha (IFNα), interferon gamma (IFNγ), nicotinamide adenine dinucleotide phosphate (NADPH), regulatory T cell (Treg), T cell receptor (TCR), T helper type 1 (Th1), T helper type 2 (Th2), toll-like receptor 7 (TLR7), tumor necrosis factor alpha (TNFα)

effector memory T cells were increased (at lower steroid doses). Transcriptomic analysis using whole transcriptome gene expression microarray profiling demonstrated that Nuclear Factor kappa B (NF-kb) signaling, inflammation and cell death-related mRNA were all suppressed while apoptosis and cell cycle transcripts were upregulated. Gene set enrichment showed upregulation of CD163, ADRB2 and IL1R2 transcripts as well as decreased NF-KB and AP-1 transcripts. Circulating cytokines were also examined with diminished secretion of inflammatory cytokines noted, including IL-1b, IL8 and TNF-related apoptosis-inducing ligand (TRAIL).

Glucocorticoids have previously been shown to upregulate both PD-1 and CTLA expression [45, 46]. Our laboratory has identified that glucocorticoids suppress the proliferation and differentiation of naïve T cells via CD28 costimulatory pathways. Interestingly, CTLA-4 inhibitors appear capable of partially rescuing T cells exposed to glucocorticoids. As CTLA-4 is the shared ligand for CD28 and CD80/86, CTLA-4 blockade would potentially allow for increased interaction of CD80/86 with CD28, thereby leading to increased T cell activation.

The effect of glucocorticoids on immune checkpoint inhibitor therapy

A chief concern in the aforementioned clinical trials examining immune checkpoint inhibitors in glioblastoma has been the relatively high incidence of steroid use in these patients. In CheckMate 143 for example, 30% of patients were receiving steroids (all $\leq$4 mg dexamethasone equivalents) at the time of immune checkpoint initiation. There were five long term survivors, none of whom had received steroids at baseline. The absence of any long-term survivors among the patients receiving steroids suggests a detrimental role. If glucocorticoids were inhibitory towards T lymphocyte activation, this effect may have predominated over that of the immune checkpoint blockade, thereby suppressing the immune system. In contrast to this are the findings from the Ivy Foundation study. In that study, steroid dose at registration did not correlate with the OS, nor did this correlate with interferon, T cell or cell cycle-associated gene expression scores. Therefore, there remains conflicting evidence as to the impact of glucocorticoids on OS in patients with glioma treated with checkpoint inhibitors.

Data in other solid tumor malignancies on the impact of glucocorticoids has likewise been mixed. Several case reports, clinical trials and systematic reviews have suggested that glucocorticoids do not reduce the efficacy of PD-1, PD-L1 or CTLA-4 directed therapy [47–51]. However, other studies have conversely suggested that glucocorticoids do indeed decrease efficacy in patients receiving immunotherapy [52, 53]. Margolin et al conducted an open label phase II study of 72 patients with melanoma metastatic to the brain and subsequently treated with ipilimumab [54]. Disease control at 12 weeks and survival among symptomatic patients on corticosteroids was 5% and 3.7 months respectively. This is in comparison to neurologically asymptomatic patients who had a disease control of 18% and OS of 7 months. Intracranial disease control among was 10% in patients on corticosteroids, compared to 24% among asymptomatic patients. Arbour et al recently reported on 640 patients with advanced NSCLC treated with PD-(L)1 blockade [55]. 14% of these patients were on $\geq$10 mg of prednisone equivalent per day within 30 days of initiating PD-(L)1 blockade. After multivariate analysis, baseline corticosteroid exposure remained significantly associated with decreased OS (HR 1.7, $p < 0.001$). Another retrospective study of 151 patients with metastatic NSCLC also suggests that corticosteroid use within 28 days of starting an immune checkpoint inhibitor is associated with poorer disease control, PFS and OS (HR 2.60, $p < 0.001$) [34]. Early use of corticosteroids in this study was noted to correlate with an increased neutrophil to lymphocyte ration (NLR), higher neutrophils and lower eosinophil counts. Interestingly, NLR $\geq$ 5 was independently associated with both early steroid usage and decreased survival indicating that this cellular ratio may in fact serve as the intermediary of immune resistance [56]. To this effect clinical tools using this biomarker to screen patients who are most likely to derive benefit from immune checkpoint inhibitors are currently under development [57].

Conclusion

The success of immunotherapy in other solid tumor malignancies, including those with intracranial metastasis, has driven renewed hope in achieving a meaningful improvement of survival for patients suffering from primary CNS tumors. However, initial clinical trials investigating immune checkpoint inhibitors in glioblastoma have been disappointing. Phase III results indicate that PD-1 blockade does not significantly benefit unselected patients with glioblastoma in the newly-diagnosed or recurrent setting. As with the initial studies of immune checkpoint inhibitors in NSCLC, identification of appropriate biomarkers remains the 'holy grail'. Such biomarkers would allow both for better patient selection, to enrich for patients most likely to respond and avoiding treatment and exposure to adverse events in those who will not. As such, there remains concern that the widespread use of glucocorticoids in patients with glioblastoma may account, in part, for a failure of immune checkpoint inhibitors to activate the immune system sufficiently as to achieve an anti-tumoral response.

There are several unanswered questions in this regard. Although there have been conflicting observations, the mounting evidence supports the position that glucocorticoids exposure during PD-(L)1 inhibition initiation decreases survival. Second, there needs to be better understanding of the appropriate "washout" and "exposure" periods whereby a patient could be tapered off of, and initiated on, glucocorticoids while still deriving clinical benefit from immunotherapy. The benefit of glucocorticoids in the treatment of certain sequela of CNS tumors, particularly cerebral edema, is undeniable, and in some patients these conditions would undoubtedly necessitate glucocorticoid treatment. However, there are other situations wherein alternative treatments such as diuretics, surgical decompression, VEGF inhibition and hyperbaric oxygen could substitute for glucocorticoid treatment and thus allow patients to receive maximal benefit from immunotherapy. Third, the exact mechanism by which glucocorticoids impair T lymphocyte activation in the setting of checkpoint blockade has not been fully elucidated. It is not clear whether this impairment is by the direct downregulation of TCR signaling by secondary modulator molecules on CD8 T cells predominantly, or other alterations of the immune milieu, such those involving T regulatory cells, tumor-associated macrophages or the tumor itself. In conclusion, key questions remain to be elicited as to the impact of glucocorticoid exposure on patient's receiving immune checkpoint therapy. The answers to these questions may provide insight for how to translate the success of these novel agents into efficacy for patients with central nervous systems tumors.

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Compliance with ethical standards

Conflict of interest WJK and MRG declare no conflict of interest.

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