

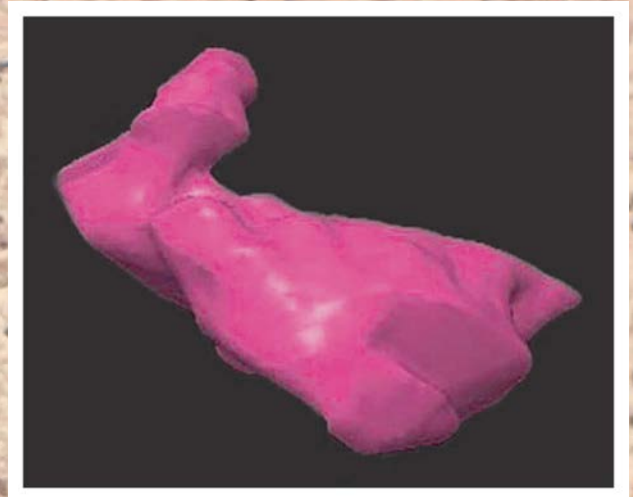
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Emerging Immune Therapeutics Targeting Glioblastoma-Mediated Immune Suppression: Dark Before the Dawn

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Emerging Immune Therapeutics Targeting Glioblastoma-Mediated Immune Suppression: Dark Before the Dawn

Shuo Xu^{1,2}, Amy B Heimberger²

Abstract: As the most common and particularly devastating primary brain malignancy, glioblastoma exerts profound immunosuppression on the anti-tumour weapons of the immune system, which also poses a tremendous obstacle to immunotherapy. By targeting glioblastoma-mediated immune suppression, enthusiasm and

confidence are accumulating based not only on the encouraging results of current clinical trials but also largely on promising preclinical findings. In this article, we summarize causes of glioblastoma-mediated immune suppression, review the current and potential approaches against several key immunosuppressive regula-

tors, and discuss the challenges and future of immunotherapy in glioblastoma treatment. **Eur Assoc NeuroOncol Mag 2013; 3 (1): 15–22.**

Key words: glioblastoma, immunosuppression, immunotherapy, clinical trials

■ Immunosuppression and Its Influence on Glioblastoma Treatment

Despite the marked advances in basic scientific research and clinical practice over the last several decades, improvements in progression-free survival (PFS) and overall survival (OS) have been modest in patients with glioblastoma – the most common and particularly devastating primary brain malignancy [1–4]. The failures of conventional glioblastoma treatments are attributed to the complex and heterogeneous tumour composition, aggressive diffuse infiltration, exuberant angiogenesis, and the tumour's capacity to escape therapies [5, 6]. Glioblastomas express a variety of tumour-associated and tumour-specific antigens such as interleukin- (IL-) 13RA, EGFRvIII, EphA2, survivin, and CMV, etc. By inducing anti-tumour immune responses, glioblastoma immunotherapy provides an alternative treatment strategy, with the theoretical advantage of tumour specificity.

Considering the presence of the blood-brain barrier, lack of lymphatic drainage, and the paucity of resident specialized antigen-presenting cells (APC) within the central nervous system (CNS), “immunological privilege” was once believed to be an inherent property of the brain. This concept has been significantly revised by the evidence of dynamic immune responses found in various physiological and pathophysiological circumstances inside the CNS [7]. In the context of glioblastoma immune responses, although there can be significant immune cell infiltration (including microglia/macrophages, lymphocytes, and dendritic cells), the anti-tumour immune responses are markedly impaired and can actually be tumour-promoting [8]. In fact, the immune responses within the glioblastoma microenvironment can be profoundly immunosuppressive and include recruitment and induction of regulatory T cells (Tregs) [9–11]; expression of immune checkpoints

(such as B7.H1/PD-L1) [12–14] and cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) [15]; down-regulation or absence of tumour-specific antigens; immunosuppressive cytokine secretion, such as transforming growth factor- (TGF-) β , IL-10, and vascular endothelial growth factor (VEGF) [16, 17]; and recruitment and skewing of tumour-supportive macrophages (M2 vs M1) [18, 19].

Immune therapeutic strategies that induce immune effector responses have demonstrated marked increases in PFS and OS in phase-II clinical trials of glioblastoma patients [20–25]. However, several of these trials have included in the enrolment criterion a requirement for gross-total resection in order to minimize glioblastoma-mediated immune suppression. Unfortunately, not all glioblastoma patients are subjected to extensive resections or may have medical contraindications. If glioblastoma-mediated immune suppression could be controlled, then theoretically, immune recognition and clearance should occur.

■ Current Clinical Trials Targeting Glioblastoma-Mediated Immunosuppression

Numerous glioblastoma immunotherapeutic clinical trials are underway, with most designed to prime/amplify the host anti-tumour immune responses rather than to abrogate or reverse glioblastoma-mediated immunosuppression. According to the ClinicalTrials.gov database (<http://www.clinicaltrials.gov/>, updated to November 2012), there are less than 10 completed or active glioblastoma immunosuppression-targeted clinical trials documented among more than 120 clinical trials related to glioblastoma immunotherapeutics. Among the limited glioblastoma immunosuppression-targeted clinical trials, most are phase-I studies evaluating the pharmacokinetic and toxicological properties of certain reagents, or exploratory studies determining the correlation between immune status modulation and drug intervention (Table 1).

TGF- β Pathway

Transforming growth factor β 2 (TGF- β 2) is a potent cytokine with multiple biological activities [26] that has been found to

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Table 1. Clinical and preclinical trial targets and results for glioblastoma-mediated immunosuppression. The Cancer Genome Atlas glioblastoma database of mRNA data (Agilent microarray, sample number: 500, updated to 11/30/2012) was used as the source of data for expression and survival evaluation, and data were analyzed through the open access cBio Cancer Genomics Portal at www.cbioportal.org. The glioblastoma tissue analyzed contains glioma cells as well as tumour-supportive stromal and infiltrating immune cells. To define the expression alternation of certain mRNAs, the z-score threshold (the number of standard deviations above the mean expression level of the selected gene) was set to ± 1 . The up-regulated (up) and down-regulated (down) percentages of the mRNAs within all available glioblastoma samples and their respective correlations with overall survival (OS)/disease-free survival (DFS) are listed.

Targets	Classification	mRNA alternation within GBM	Overall survival correlation	Disease free survival correlation	Therapeutic strategy	Therapeutic agents	ClinicalTrial.gov access #	Phase	Results
TGF β	Immunosuppressive cytokine	Up: 11.2 % Down: 16.0 %	Up: ns Down: positive (p = 0.027)	Up: ns Down: positive (p = 0.012)	Anti-sense oligonucleotide; antibody blockade	Trabedersen (AP 12009) GC1008	NCT00431561 NCT00761280 NCT01472731	II/III I	Survival benefit for a sub-group of GBM patients (phase II)
TGFBRI	Immunosuppressive cytokine receptor	Up: 7.8 % Down: 13.0 %	Up: ns Down: ns	Up: ns Down: ns	Small molecule inhibitor	LY2157299	NCT01220271	I/II	–
IL-2R α (CD25)	Treg marker	Up: 25.0 % Down: 11.6 %	Up: ns Down: ns	Up: negative (p = 0.0048) Down: ns	Antibody blockade	Basiliximab Daclizumab	NCT00626015 NCT00626483	I/II	Well-tolerated; peripheral Treg reduction
VEGF-A	Immunosuppressive cytokine	Up: 15.6 % Down: 20.6 %	Up: ns Down: ns	Up: negative (p = 0.016) Down: ns	Antibody blockade	Bevacizumab	NCT01091792	0	–
CTLA-4	Immune checkpoint	Up: 12.2 % Down: 12.0 %	Up: ns Down: ns	Up: ns Down: ns	Antibody blockade	Ipilimumab Tremelimumab	–	–	–
PD-1	Immune checkpoint	Up: 8.4 % Down: 12.4 %	Up: ns Down: ns	Up: ns Down: ns	Antibody blockade	MDX-1106	–	–	–
PD-L1	Immune checkpoint	Up: 8.8 % Down: 18.0 %	Up: ns Down: positive (p = 0.044)	Up: negative (p = 0.063) Down: positive	Antibody blockade	MDX1105-01	–	–	–
STAT3	Transcription regulator	Up: 13.0 % Down: 17.0 %	Up: negative (p = 0.0096) Down: ns	Up: negative (p = 0.042) Down: ns	Small molecule inhibitor	WPI066	–	–	–
IDO	Immunosuppressive enzyme	Up: 9.8 % Down: 1.6 %	Up: ns Down: ns	Up: ns Down: ns	Small molecule inhibitor	1-MT	–	–	–
Arg-1	Immunosuppressive enzyme	Up: 14.0 % Down: 17.8 %	Up: ns Down: ns	Up: ns Down: ns	Small molecule inhibitor	nor-NOHA	–	–	–

ns: non-significant; positive: the OS/DFS of an altered gene set is larger than for unaltered ones, p < 0.05; negative: the OS/DFS of an altered gene set is smaller than for unaltered ones, p < 0.05.

be over-expressed in more than 90 % of high-grade gliomas [27–29], making it an attractive target for glioblastoma treatment. Inhibition of TGF- β 2 in tumour tissue leads to reversal of tumour-induced immune suppression as well as inhibition of tumour growth, invasion, and metastasis [30, 31]. Trabedersen (AP 12009) is a synthetic antisense phosphorothioate oligodeoxynucleotide complementary to the human TGF- β 2 mRNA, whose safety and efficacy has been shown through various pharmacokinetic and toxicology studies, both in vitro and in vivo, as well as in phase-I/II dose-escalation studies [32–34]. The overall outcome was negative for the pre-specified primary endpoint in the randomized phase-IIb study comparing a dose of either 10 μ M or 80 μ M of trabedersen with standard chemotherapy in a cohort of 145 patients with recurrent or refractory anaplastic astrocytoma or glioblastoma (Trial Registration: ClinicalTrials.gov NCT00431561). Nevertheless, survival at 2 and 3 years for glioblastoma patients in a small subgroup (≤ 55 years old, with an initial Karnofsky Performance scale score of > 80) who received 10 μ M trabedersen was 3-fold higher than for those receiving chemotherapy [35]. Despite the controversy over the interpretation of the clinical trial data [36, 37], a multinational phase-III study entitled SAPHIRE was underway investigating the efficacy and safety of 10 μ M trabedersen compared with standard chemotherapy in adult patients with confirmed recurrent or refractory anaplastic astrocytoma or secondary glioblastoma, but it was terminated due to slow patient recruitment (Trial Registration: ClinicalTrials.gov NCT00761280).

In addition to trabedersen, several other drugs targeting the TGF- β pathway are now being tested in phase-I/II clinical trials. For instance, GC1008 is an antibody capable of neutralizing TGF- β , and a phase-II clinical trial is open to determine its safety, tolerability, pharmacokinetics, and pharmacodynamics in primary malignant glioma patients (Trial Registration: ClinicalTrials.gov NCT01472731). LY2157299 is another new small molecule drug antagonizing the TGF-

β receptor I/II kinase [38] that is being tested in a phase-I/II clinical trial with temozolomide-based radiochemotherapy in patients with newly diagnosed malignant glioma (Trial Registration: ClinicalTrials.gov NCT01220271).

CD25/IL-2R α

An increase in the Treg fraction and amount of Treg infiltration has been verified as an immunosuppression-related characteristic in the peripheral blood and tumour tissue of glioma patients, especially in high-grade gliomas [9–11]. Expression of alpha chain of the IL-2 receptor (CD25/IL-2R α) not only serves as a Treg phenotypic marker, but it also empowers the Treg to induce IL-2 cytokine deprivation-mediated apoptosis of effector T cells [39, 40]. Preclinical studies conducted by Dr John Sampson's group have shown that systemic Treg cell depletion with anti-mouse CD25 mAb (Clone PC61) can significantly extend survival in an SMA-560 glioma syngeneic mouse model system [41]. Inspired by this, several anti-CD25-based phase-I/II clinical trials in glioblastoma patients have been initiated with basiliximab (Trial Registration: ClinicalTrials.gov NCT00626015) and daclizumab (Trial Registration: ClinicalTrials.gov NCT00626483). Both basiliximab and daclizumab are mouse-human chimeric monoclonal antibodies that specifically block IL-2-to-IL-2R α binding and were initially designed and approved by the US Food and Drug Administration (FDA) to prevent acute rejection after organ transplantation. In contrast to the PC61 antibody which depletes murine CD25⁺ T cells, basiliximab and daclizumab act through a non-depleting mechanism [42]. The purpose of the current clinical trials is to study the safety and combinatorial approaches for patients with resected glioblastoma. Previous attempts to selectively eliminate Tregs with denileukin diftitox (ONTAK, a fusion protein of diphtheria toxin and IL-2) and LMB-2 (a fusion protein of an anti-IL-2R α monoclonal antibody and exotoxin) resulted in mixed success and off-target limitations [43]. Of note, a recently published randomized placebo-controlled pilot study indicates that daclizumab treatment is well-tolerated with no symptoms of autoimmune toxicity and resulted in a significant reduction in the frequency of circulating CD4⁺FoxP3⁺ Tregs cells relative to saline controls [44], indicating that further large-scale studies are warranted.

VEGF

Dynamic endothelial cell proliferation and abnormal vessel formation are among the major characteristics of glioma pathology, which are mainly driven by elevated VEGF signalling in the tumour microenvironment [45, 46]. Via ligand interaction with VEGFR, VEGF initiates PI3K/Akt, MEK/Erk, and other signalling pathways, which trigger endothelial cell adhesion, migration, and growth [41]. Along with its proangiogenesis effect, VEGF induces immunosuppression and other regulatory functions to promote glioma progression [48, 49]. The anti-VEGF monoclonal antibody bevacizumab has been approved by the FDA for the treatment of glioblastoma since 2009. Although there is still considerable debate regarding the overall survival benefit of bevacizumab and whether or not it induces more infiltrative glioma recurrence in some patients [50], it has been shown to prolong PFS and control peritumoural oedema [51]. A phase-0 clinical trial has been opened for newly diagnosed glioblastoma patients to evaluate

the respective roles of radiotherapy, temozolomide (TMZ), and bevacizumab on Treg shift and modulation of the immune system (Trial Registration: ClinicalTrials.gov NCT01091792).

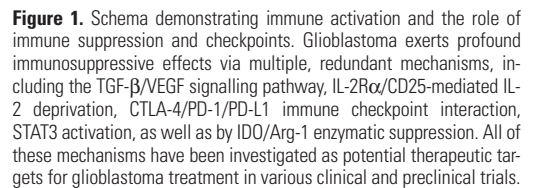
Several studies have suggested that bevacizumab may improve immunological responses by abrogating VEGF-induced inhibition on dendritic cells, reconstitution of the lymphocyte compartment, modulation of cytokine secretion, and decreasing the Treg fraction [52–55]. Consequently, a phase-II clinical trial of EGFRvIII peptide in combination with bevacizumab (Trial Registration: ClinicalTrials.gov NCT00671970) has been initiated. However, human glioblastoma tumours and their murine xenografts have been shown to have markedly increased expression of immune suppressive signal transducer and activator of transcription 3 (STAT3) upon failing to respond to bevacizumab therapy [56], suggesting that sustained use of bevacizumab may hinder immune therapeutic approaches. To date, no published studies have evaluated the synergistic activity of bevacizumab and immunotherapy in murine glioma model systems.

■ Proposed Therapeutics Targeting Glioblastoma-Mediated Immunosuppression

Unfortunately, most present clinical trials abrogating glioblastoma-mediated immune suppression suffer from small sample size, administration optimization, and bias induced by patient selection. It is too early to draw conclusions regarding therapeutic success or failure of these approaches based on these limited clinical trials. However, the observations of clinical symptom alleviation, survival advantage in select patients, low toxicity, and supplementary effect to standard treatments have generated enthusiasm. Moreover, with the identification of key hubs in glioblastoma-mediated immunosuppression, preclinical findings have indicated broad and encouraging opportunities to enhance anti-tumour immunity. Some of these targets have been well-tested in other types of cancer. Given extensive preclinical data and target expression analysis, we are clearly on the precipice of evaluating therapeutic efficacy of several approaches targeting glioblastoma-mediated immune suppression.

CTLA-4

T-cell activation is initiated through antigens presented in the context of the major histocompatibility complex (MHC) to the T-cell receptor (TCR; Figure 1). The T-cell response status and amplitude are regulated by the balance between co-stimulatory and inhibitory signals [57, 58]. Specifically, CD28 signalling by binding to co-stimulatory molecules such as CD80 (B7.1) and CD86 (B7.2) provides the second signal for T-cell proliferation, resulting in pro-inflammatory cytokine secretion and cytotoxic killing. On the contrary, inhibitory signals, or so-called immune checkpoints, down-regulate the activation signal and induce T-cell inactivation, anergy, and apoptosis to maintain immune homeostasis. In the case of glioblastoma, these immune checkpoint haemostatic mechanisms can be up-regulated inappropriately by the tumour to limit immune recognition and elimination of the malignancy [13, 14, 59]. Well-characterized checkpoint molecules that play a role in tumour immune suppression include the cyto-



An anti-human CTLA-4 antibody, ipilimumab, has recently been approved by the FDA for the treatment of stage-IV melanoma [69], which begets enthusiasm for CTLA-4 blockade in the treatment of other cancers. Furthermore, radiographic responses have been observed in melanoma patients with CNS metastasis treated with ipilimumab. In preclinical studies with the murine SMA-560 intracranial glioma model, systemic administration of anti-CTLA-4 Ab (Clone 9H10) conferred long-term survival in 80 % of treated mice, without eliciting experimental allergic encephalomyelitis. CTLA-4 blockade re-established a normal CD4 fraction, restored T-

Programmed cell death protein 1 (PD-1) is another immune checkpoint receptor expressed by activated T cells. While CTLA-4 regulates T cells at the initial stages of activation, PD-1 dampens the immune response during later stages of T-cell activation by interacting with its ligands, programmed cell death protein 1 ligand 1 (PD-L1/B7-H1) and PD-L2 (B7-DC), expressed on hematopoietic cells, including antigen-presenting cells (APCs), T cells, B cells, macrophages, and stromal cells [70–73]. Its ligand PD-L1 is also expressed by glioblastoma cells, including the cancer stem cells [12–14, 74], indicating this PD-1/PD-L1 pathway is a relevant thera-

peutic target for glioblastoma patients. In addition, PD-1 participates in the regulation of humoral immunity [75, 76] and innate immunity [77], suggesting that this mechanism might exert an even broader regulatory effect on immune responses than CTLA-4. Of note, PD-1 is highly expressed on Tregs and enhances Treg proliferation [78].

Inspiringly, the results from 2 phase-I clinical trials (Trial Registration: ClinicalTrials.gov NCT00730639 and NCT00729664) using monoclonal antibodies that antagonize the PD-1/PD-L1 pathway (MDX-1106 for PD-1 and MDX1105-01 for PD-L1) were reported recently for several tumour types, including advanced melanoma, non-small-cell lung, prostate, renal, colorectal, ovarian, pancreatic, gastric, and breast cancers. Antibody-mediated blockade of the PD-1/PD-L1 pathway induced durable tumour regression (objective response rate of 6–28 %) without severe toxicity (grade-3 or -4 drug-related adverse event rate of 9–14 %) [79, 80]. Based on these encouraging results, the strategy of blocking the PD-1/PD-L1 pathway might provide a new benchmark for anti-cancer immunotherapy [81, 82]. Given the PD-L1 expression on glioma cells, the established role of the PD-1/PD-L1 pathway in glioma immunosuppression, and the relationship between PD-L1 expression on tumour cells and the anti-PD-1 therapy objective response [80], a clinical trial of these agents in glioblastoma patients, including stratification based on expression, warrants consideration. Preclinical studies have demonstrated prolonged survival in the intracranial GL261 glioma C57/BL6 murine model using the combination of anti-PD-1 therapy and radiation therapy when compared with either modality alone [83].

STAT3

STAT3 is a key transcription factor that drives the fundamental components of malignancy and invasion (including in gliomas) and is considered a master transcriptional regulator of tumorigenesis [84]. Although not mutated in glioma, STAT3 is phosphorylated and therefore activated in nearly all gliomas. Many growth factors and cytokines, including IL-6 that is expressed in the CNS, activate Jak2, which subsequently activates STAT3 by phosphorylation of the tyrosine residue in the transactivation domain. Phosphorylated STAT3 (p-STAT3) then translocates into the nucleus and induces a variety of effector molecules. STAT3 is frequently over-expressed in tumour cells, including gliomas [85], and drives tumorigenesis by preventing apoptosis (by increasing survivin, Bcl-xl, and Mcl-1 expression) and enhancing proliferation (by increasing c-Myc and cyclin D1/D2 expression), angiogenesis (by increasing VEGF and hypoxia-inducible factor- [HIF-] 1 α expression), and invasiveness (by increasing matrix metalloproteinase- [MMP-] 2/9 expression) [86, 87] – including specifically within gliomas [19, 88–90].

STAT3 has also been strongly implicated as a key regulator of immunosuppression in patients with cancer [91]. The p-STAT3 pathway is activated in the immune cells, especially the immune cells that reside within the tumour microenvironment [92], which down-regulates the anti-tumour immune responses of the immune cells. Others have shown that the up-regulation of p-STAT3 reduces the expression of MHC II, CD80, CD86, and IL-12 in dendritic cells, rendering them unable to stimulate T cells and generate effective anti-tumour

immunity [92] and that STAT3 is a transcriptional regulator of FoxP3 expression in Tregs [93]. We have shown that human glioblastoma can specifically polarize macrophages via the STAT3 pathway toward an immunosuppressive and tumour-supportive M2 phenotype [19] that then contributes to angiogenesis and tumour invasion and is a negative prognosticator for long-term survival in genetically engineered murine model systems of glioma [18].

Recent studies have indicated that STAT3 is essential for glioma cancer stem cell (gCSC) maintenance [94, 95]. gCSCs have the capacity for self-renewal, and their main feature is their ability to initiate a tumour in mice. gCSCs have been shown to recapitulate the characteristics of glioblastoma [96], and they are also believed to confer the resistance to chemotherapy and radiation observed in glioblastoma [97, 98]. They are also profoundly immune suppressive and can express CTLA-4 and B7-H1 [99]. Moreover, gCSCs elaborate a variety of immune suppressive cytokines such as galectin-3 and TGF- β that inhibit T-cell proliferation and activation, induce FoxP3⁺ Tregs, trigger T-cell apoptosis, and induce macrophages/microglia to become polarized to the tumour-supportive p-STAT3-expressing M2 phenotype [19, 100]. Many think that without targeting the gCSC population of cells therapeutically, malignant gliomas will continue to persist and recur.

WP1066 blocks the nuclear translocation of p-STAT3 into the nucleus [101, 102] and achieves excellent penetration into the CNS. Therapeutic efficacy with WP1066 has been demonstrated against head and neck carcinoma [103], pancreatic cancer [104, 105], bladder cancer [106], B-cell non-Hodgkin's lymphoma and myeloma [107], and chronic myelogenous leukaemia [108]. WP1066 has demonstrated therapeutic efficacy against metastatic [109] and established CNS melanoma in murine models [110]. The therapeutic effects of WP1066 can be partially ablated with in vivo depletions of the CD4⁺ and CD8⁺ population or by implanting B16 melanoma or GL261 glioma in nude mouse model systems, indicating that part of the therapeutic effect of WP1066 is immunologically mediated [110]. WP1066 has demonstrated therapeutic efficacy against subcutaneously implanted U-87 MG cells [89] and in 2 distinct Ntv-A murine models of gliomas, those induced by RCAS-PDGFB + RCAS-bcl-2 [89] and by RCAS-PDGFB + RCAS-STAT3 [88].

We have also demonstrated that STAT3 blockade with WP1066 can significantly modulate tumour-mediated immune suppression. Specifically, WP1066 can induce the expression of co-stimulatory molecules on peripheral macrophages and tumour-infiltrating microglia ex vivo from glioblastoma patients. WP1066 treatment of the peripheral blood from glioblastoma patients who are immunologically anergic resulted in marked production of pro-inflammatory cytokines including IL-2, IL-4, IL-12, and IL-15. STAT3 blockade with WP1066 was sufficiently potent to induce proliferation of effector T cells from glioblastoma patients who were refractory to CD3 stimulation, and mechanistically, this was found to be secondary to the activation of ZAP-70 in the T cells and inhibition of Tregs [111]. Furthermore, the immunosuppressive properties of GSCs were markedly diminished when the GSCs were treated with either siRNA targeting STAT3 or with WP1066

[19, 100]. Specifically, the gCSC-mediated effects of inhibiting T-cell proliferation and inducing Tregs were markedly diminished when the STAT3 pathway was blocked in the gCSCs. Furthermore, WP1066 could inhibit the gCSC-conditioned medium polarization of the macrophage/microglia to the M2 immune suppressive, tumour-propagative phenotype, and inhibited the secretion of the immunosuppressive cytokines interleukin-10 (IL-10) and TGF- β 1 by the M2 macrophages/microglia. Combined, these data indicate that WP1066 can reverse both innate and adaptive tumour-mediated immune suppression. Cumulatively, these data provide compelling rationale for therapeutically targeting the STAT3 pathway. It is anticipated that WP1066 will be entering clinical trials for glioblastoma patients within the next year.

Indoleamine 2,3-Dioxygenase

Indoleamine 2,3-dioxygenase-1 (IDO) is an inducible enzyme that converts tryptophan to kynurenine. It has been demonstrated to be a potent immunosuppressive regulator expressed by cancer cells as well as by infiltrating immune cells, including dendritic cells and myeloid-derived suppressor cells (MDSCs) [112, 113]. Up-regulated by high IFN- γ and TGF- β within the tumour microenvironment, IDO utilizes various mechanisms to induce local immunosuppression, including depletion of the essential amino acid tryptophan, tryptophan toxic metabolite accumulation, NF- κ B signalling activation that leads to effector T-cell suppression and Treg induction, up-regulation of inhibitory TGF- β and CTLA-4, and modulation of the maturation and/or function of dendritic cells [114]. In addition, IDO expression induces Treg recruitment and inhibition of T-cell-mediated glioma immunity, which suggests a critical role for IDO-mediated immunosuppression in glioblastoma [115]. Recently, Dr Maciej Lesniak's group demonstrated that IDO down-regulation in glioma predicts a better prognosis in both mouse models and human patients.

The potential of IDO as a therapeutic target in glioblastoma treatment has been investigated in several preclinical studies [116, 117]. The agent 1-methyl-tryptophan (1-MT) is a competitive inhibitor for IDO [118]. Several phase-I clinical trials based on 1-MT are underway in advanced solid tumours, including breast, lung, and pancreatic cancers as well as melanoma. Although there is no current clinical trials of IDO inhibition in glioblastoma patients, this is also a potential approach in glioblastoma treatment. Of note, selective inhibition of IDO is expected to exert a therapeutic effect without significant side effects because IDO is an inducible enzyme [119].

Arginase-1

As a marker of tumour-supporting M2 macrophages [120, 121] and MDSCs [122], arginase-1 (Arg-1) has been shown to exert immunosuppressive effects through the consumption of L-arginine, a critical cofactor for sustained T-cell activation [123, 124]. Increased Arg-1 level and activity are observed in the plasma obtained from patients with glioblastoma, suggesting the functional dysregulation of circulating neutrophils and MDSCs. Interestingly, *in vitro* T-cell suppression induced by peripheral blood mononuclear cells (PBMCs) from glioblastoma patients could be reversed by the specific Arg-1 inhibitor nor-NOHA or by arginine supplementation [125, 126].

Other Targets

Other potential targets for glioblastoma-mediated immune suppression include IL-10 [127, 128], GTR [129, 130], and inducible nitric oxide synthase (iNOS) [131]. Although there are no current clinical trials in glioblastoma patients targeting these molecules, agents targeting these are being evaluated in other types of cancers and have shown promise.

Perspectives

A fundamental challenge for targeting glioblastoma-mediated immune suppression is tumour heterogeneity. This feature undoubtedly contributes to the tumour's aggressiveness and poses a tremendous obstacle to the glioblastoma treatment, including immunotherapy [132]. With the plethora of immunosuppressive mechanisms described to date and the possibility of more yet to be identified, multiple, redundant mechanisms are utilized by glioblastoma to escape host immune attack. It would be anticipated that the targeting of one immunosuppressive mechanism would result in the rapid appropriation of alternative mechanisms. A key reason for immunotherapeutic failure is the dynamic immunoediting and immune escape within the glioma environment [133]. It is very likely that an optimized arsenal of multiple immunotherapeutics targeting corresponding mechanisms and used in combination with other standard approaches will be necessary to exert a sustained suppression of malignancy.

Alternatively, agents could be used that target networks of immune suppression. MicroRNAs (miRNAs) are a group of small non-coding RNA molecules, which post-transcriptionally regulate genes by binding to the 3'-untranslated region (UTR). Interestingly, one single miRNA can regulate multiple target genes and vice versa. Because miRNAs have been demonstrated to modulate tumour cell proliferation and apoptosis and to act as oncogenes or tumour-suppressor genes, the connection between tumour-mediated immune suppression and miRNAs has yet to be explored. Our preliminary studies have shown that miRNA dysregulation is present in the glioma microenvironment and that it participates in the glioma-mediated immunosuppression. Moreover, selective miRNA administration or inhibition can exert potent anti-glioma therapeutic effects via the immune system and can target multiple immunosuppressive mechanisms simultaneously (unpublished data, 2012). Thus, these miRNAs may represent a new class of immune therapeutics that have the potential to modulate multiple mechanisms of glioblastoma-mediated immune suppression.

Conclusions

Complex and profound glioblastoma-associated immunosuppression abrogates anticancer immune responses. The limited success of current glioblastoma immunotherapy further provokes the necessity for new approaches. Recent preclinical findings targeting the key hubs of glioblastoma-mediated immunosuppression along with lessons learned from the other cancers indicate broader and more encouraging opportunities. Despite the tremendous challenges and difficulties ahead, multiple-target or personalized glioblastoma-mediated immune suppression therapeutic targeting combined with other

standard approaches will undoubtedly bring the dawn to glioblastoma treatment.

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Conflict of Interest

The authors state that no conflicts of interest exist.

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