Vaccine-based immunotherapeutic approaches to gliomas and beyond

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Abstract | Astrocytic and oligodendroglial gliomas are intrinsic brain tumours characterized by infiltrative growth and resistance to classic cancer therapies, which renders them inevitably lethal. Glioblastoma, the most common type of glioma, also exhibits neoangiogenesis and profound immunosuppressive properties. Accordingly, strategies to revert glioma-associated immunosuppression and promote tumour-directed immune responses have been extensively explored in rodent models and in large clinical trials of tumour immunotherapy. This Review describes vaccination approaches investigated for the treatment of glioma. Several strategies have reached phase III clinical trials, including vaccines targeting epidermal growth factor receptor variant III, and the use of either immunogenic peptides or tumour lysates to stimulate autologous dendritic cells. Other approaches in early phases of clinical development employ multipeptide vaccines such as IMA-950, cytomegalovirus-derived peptides, or tumour-derived peptides such as heat shock protein-96 peptide complexes and the Arg132His mutant form of isocitrate dehydrogenase. However, some preclinical trial data suggest that addition of immunomodulatory reagents such as immune checkpoint inhibitors, transforming growth factor-β inhibitors, signal transducer and activator of transcription 3 inhibitors, or modifiers of tryptophan metabolism could augment the therapeutic activity of vaccination and overcome glioma-associated immunosuppression.

Throughout the past century, pivotal research issues in oncology have included the extent to which immunosuppression is a risk factor for the development of cancer, and the extent to which cancer induces immunosuppression. Accordingly, immunosuppression has been extensively studied in brain tumours such as glioma, which commonly kills affected patients by locally destructive growth rather than by systemic metastasis. Multiple cellular interactions and pathways that might potentially mediate glioma-associated immunosuppression have been identified[1,2] (FIG. 1).

Several decades ago, researchers also recognized that gliomas in the brain promote systemic immunosuppression to a certain degree[3], although such immunosuppression is not associated with an increased risk of opportunistic infections. Tumours growing in essentially immunocompromised environments such as the brain would not be expected to derive an advantage from inducing additional immunosuppression. Moreover, even glioblastomas — the most common and most malignant gliomas — seem to be largely incapable of seeding outside the CNS[4]. This observation has been

attributed, at least partially, to immune defence mechanisms that operate only outside the brain. However, experimental or clinical data supporting the existence of efficient anti-glioma immune responses in the periphery, but not inside the CNS, has remained very challenging to obtain.

One of the most remarkable features of gliomas is the observation that these tumours develop much more frequently in elderly individuals (aged ≥60 years), and that outcome is substantially worse in this age group than in younger individuals[5]. Molecular profiling has enabled the characterization of distinct subtypes of glioblastoma that are most common in children and adults up to 40 years of age[6]. In the population of adults with glioblastoma, the small prognostically favourable subgroup of tumours with isocitrate dehydrogenase (*IDH*) gene mutations is virtually absent in patients aged ≥60 years[7]. By contrast, high-throughput studies of the three most common *IDH* wild-type glioblastoma subtypes in adults (namely, receptor tyrosine kinase (RTK) 1, RTK 2, and mesenchymal) show few differences at the genomic, transcriptomic or epigenetic level between tumours

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Key points

- Glioblastoma is the paradigm of tumour-associated immunosuppression
- Several glioma-specific peptide vaccines, with or without dendritic cell support, are in late clinical development
- Vaccines can be combined with agents that nonspecifically boost immune responses, such as immune checkpoint inhibitors or TGFβ pathway inhibitors
- Standardization of clinical trial conduct might facilitate progress in this challenging field of oncology

from elderly individuals (>65 years) and those from younger patients[6,8]. These data suggest that the status of the host, rather than the molecular make-up of the tumour, might to a large extent determine the outcome of these types of cancer.

In summary, the clinical relevance of glioma-associated immunosuppression remains debated. Nevertheless, the past 5 years have seen the clinical translation of various cancer immunotherapies, some of which are glioma-specific. Several of these strategies involve innovative vaccination strategies that are challenging, in terms of both conceptional design and logistical conduct, to implement in the clinic (FIG. 2). Immediate conceptual challenges include the choice of antigen to be targeted, the selection of patients on the basis of tumour biomarkers and immune markers (such as HLA status), and the timing of assessment of the status of such markers. Further conceptual challenges involve the integration of immunotherapy into post-surgical treatment schedules (such as tapering of corticosteroids), the wound healing process, and the initiation of radiotherapy and chemotherapy, which all occur within a few weeks. Accordingly, logistical challenges include the need for rapid testing of biomarkers (which might require shipment of biological samples), generation of the vaccine at a remote location, and delivery of the vaccine back to the hospital setting where the patient will be treated.

In this article, we review the experimental evidence supporting the development of vaccine-based immunotherapies for patients with glioma and the clinical experience gathered so far with several such approaches. We also outline current ideas about how to improve the clinical results achieved with glioma vaccines by overcoming specific limitations of current immunotherapies, including efforts to antagonize glioma-associated immunosuppression.

Data from animal models

The use of vaccination to induce immune responses against gliomas has been assessed extensively in rodent models. However, the few available syngeneic mouse glioma models do not fully reflect the biology of human glioma tumours because cell-line-based models do not reproduce the heterogeneity typical of the human disease, and genetically engineered models are, by their nature, a simplification. Furthermore, important differences between mouse and human immune systems must be considered when findings from preclinical models are translated to patients in the clinic[9]. Another important consideration in experimental studies is the timing of vaccination in relation to that of tumour cell inoculation: the longer the gap between initiation of vaccination and tumour cell inoculation, the more potentially relevant are the resulting observations for patients with unresected tumours. Conversely, vaccination soon after tumour cell inoculation (as is typical in rodent studies) might resemble the clinical scenario in patients with recently resected tumours limited to microscopic disease. Despite their limitations, however, preclinical models do provide important information that helps to predict whether glioma vaccines, alone or in combination with other drugs, will have antitumour activity in the clinical setting.

One of the most frequently used mouse glioma models is based on the GL-261 cell line, which was generated by treatment with the chemical carcinogen methylcholanthrene. These cells are usually implanted stereotactically into the brains of syngeneic C57BL/6 mice, where they produce immunogenic tumours[10]. A spontaneous glioma that developed in a VM/Dk mouse has been used to generate several syngeneic mouse glioma cell lines[11,12], which are increasingly used for immunotherapy studies and have the potential advantage over GL-261 that cancer was not chemically induced[13]. A popular genetic approach uses the RCAS-TVA retroviral gene-transfer system, which can induce syngeneic tumours even in outbred mice[14]. A popular transgenic model was created by inactivation of p53 in conjunction with loss of the *Nf1* gene (encoding neurofibromin)[15].

One simple approach to tumour vaccine development is to modify glioma cell lines *ex vivo* and to use the modified cells as a vaccine against the parent tumour in the mouse brain. For example, the growth of intracerebral SMA-560 gliomas was inhibited by peripheral vaccination using irradiated SMA-560 cells engineered to express MICA (MHC class I polypeptide-related sequence A)[16]. MICA is a ligand for NKG2D (NKG2-D type II integral membrane protein), an immunoreceptor expressed on T cells and natural killer (NK) cells that stimulates cellular immune responses[17]. Other studies have employed cytokine-transduced glioma cells as a vaccine, most using granulocyte–macrophage colony-stimulating factor (GM-CSF)[18,19]. The efficacy of vaccines derived from glioma cells might be enhanced by

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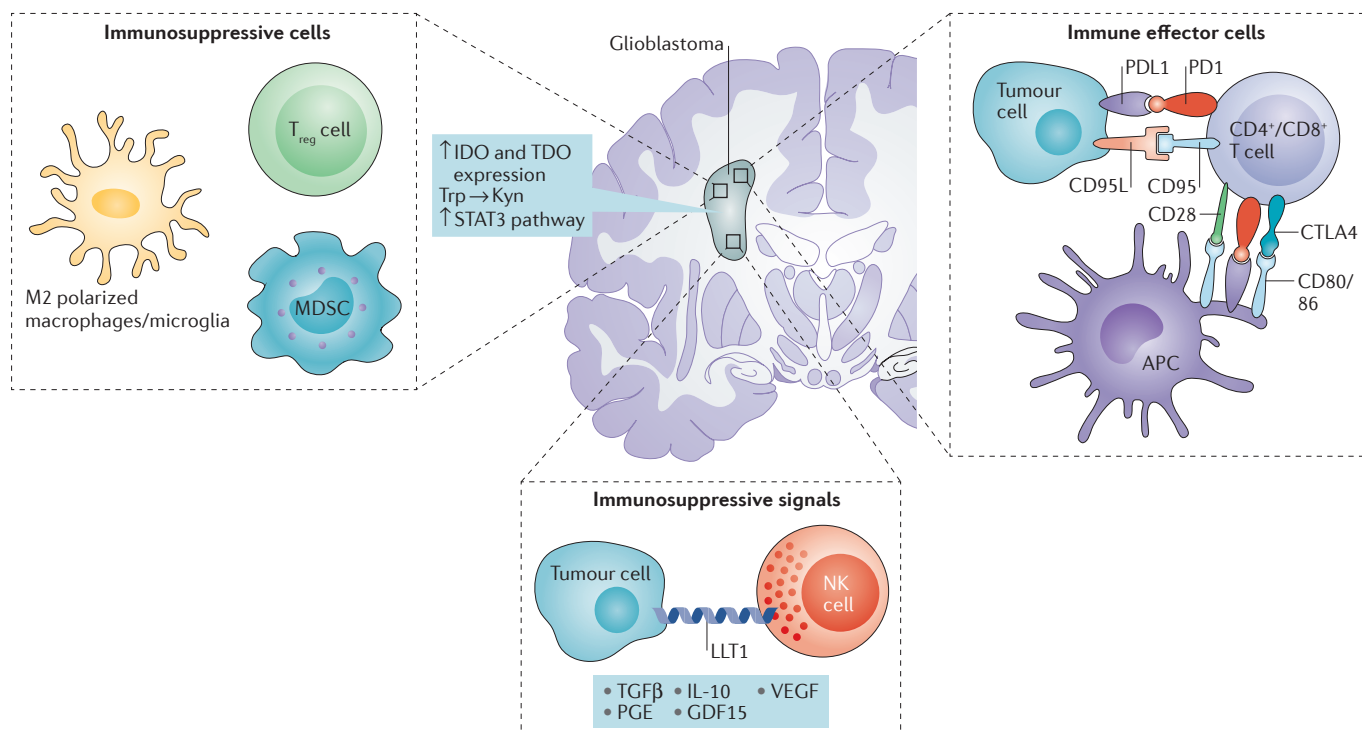
Figure 1 | **Glioma-associated pathways of local and systemic immunosuppression.** Membrane-bound and soluble mediators of glioma-associated immunosuppression both attract and promote the expansion of populations of immunosuppressive cells, and interfere with the generation of antitumour immunity at the T cell–antigen-presenting cell (APC) interface. CTLA4, cytotoxic T cell antigen 4; GDF15, growth and differentiation factor 15; IDO, indoleamine 2,3-dioxygenase; Kyn, kynurenine; LLT1, lectin-like transcript 1; MDSC, myeloid-derived suppressor cell; NK, natural killer; PD1, programmed cell death protein 1; PDL1, programmed cell death 1 ligand 1; PGE, prostaglandin E2, STAT3, signal transducer and activator of transcription 3; TDO, tryptophan 2,3-dioxygenase; TGFβ, transforming growth factor β; T_{reg} cell, regulatory T cell; Trp, tryptophan.

culturing the source cells under hypoxic conditions: vaccination with lysates from GL-261 cells cultured in 5% O_2 caused an increase in cytotoxic T-cell proliferation, tumoricidal function, and trafficking to the tumour site[20], although the mechanisms underlying this effect remained obscure.

Vaccines based on dendritic cells (DCs) have been assessed for approximately two decades in glioma-bearing mice, with variable success[21]. Effective vaccines have been prepared using DCs pulsed with tumour-specific peptides, tumour lysate, or vectors encoding putative tumour antigens[22–25]. DC-based vaccination might hold particular promise when the tumour stem cell compartment is used as a source of antigen: in terms of mounting immune responses against orthotopic gliomas, vaccines based on DCs pulsed with a lysate derived from mouse glioma cells were more effective if these glioma cells had stem cell properties[26].

Peptide-based vaccines, alone or in combination with therapies aimed at overcoming glioma-associated immunosuppression, have also been investigated in immunocompetent mouse models. For example, an antibody to transforming growth factor-β (TGFβ) augmented the efficacy of vaccination with two peptides, GARC-1$_{77-85}$ and EphA2$_{671-679}$, derived from candidate glioma-associated antigens[27].

Some evidence suggests that vaccines are more effective when given in combination with molecularly targeted therapies. For example, administration of a vaccine derived from DCs pulsed with autologous tumour lysate in combination with antibodies targeting programmed cell death protein 1 (PD1) prolonged the survival of glioma-bearing mice, whereas no such effect was observed with either treatment alone[28]. Similarly, the efficacy of a vaccine consisting of irradiated GL-261 cells expressing GM-CSF was improved by co-treatment with antibodies targeting cytotoxic T cell antigen 4 (CTLA4)[29].

The vast majority of preclinical studies focusing on vaccination in glioblastoma have limitations that impede translation of their findings to the clinic. Specifically, treatment was started very early in relation to the time of tumour cell inoculation, suggesting that vaccine treatment was initiated during immunological priming rather than during the chronic immune homeostasis stage, when treatment is typically initiated in patients with glioblastoma. Moreover, highly immunogenic tumours such as GL-261 were studied, and subcutaneous instead of intracranial tumour cell inoculation was often used, perhaps because researchers erroneously assumed that these two methods would be equally informative[30]. Finally, the age (and thus immune status) of vaccinated mice might not be comparable to that of most
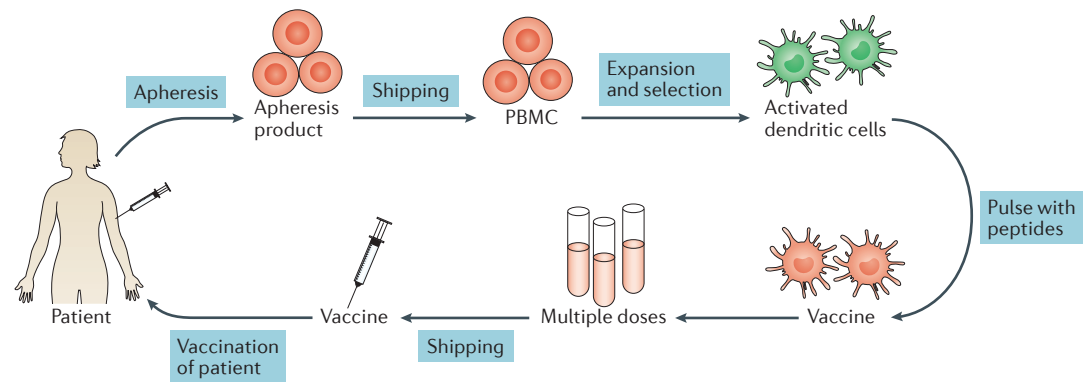
Figure 2 | **Logistical requirements for autologous immune or immune cell-based vaccination for glioblastoma.** Patients undergo apheresis to enable generation of a dendritic cell-based vaccine using peptides derived from tumour-associated antigens. Multiple doses of the vaccine can be produced and used for the patient's treatment. PBMC, peripheral blood mononuclear cell.

patients with glioblastoma. Nonetheless, despite these limitations, mouse models of glioma can lead to a more comprehensive understanding of immune system function, as well as the pitfalls associated with vaccination attempts.

Data from clinical trials

A summary of completed clinical trials of vaccination therapy for gliomas is provided in TABLE 1. Ongoing or planned trials are summarized in TABLE 2. Current approaches to vaccine-based immunotherapy for glioma exhibit variable degrees of complexity, primarily resulting from selection of the target antigen(s) and the decision whether or not to use autologous, patient-derived immune cells to generate the vaccine (which commonly requires monocyte apheresis and *ex vivo* maturation into DCs). Selection of the target antigen ranges from approaches targeting a single tumour-specific mutant protein such as isocitrate dehydrogenase (IDH) Arg132His (IDH^{R132H}) or epidermal growth factor receptor (EGFR) variant III (EGFRvIII)[31,32], to approaches targeting a predefined panel of tumour-associated antigens (such as ICT-107)[33] or a personalized panel of tumour-associated antigens selected by genome profiling from a limited pool of targets (as used in the GAPVAC trial)[34]. Unbiased antigen-selection approaches using undefined tumour-derived peptides (HSPPC-96) or whole-tumour-cell lysates (DCVax) have also been used[35,36].

Vaccines that target defined peptides (as opposed to those targeting uncharacterized proteins or using unbiased antigen selection) are probably less prone to unexpected adverse events, such as induction of tolerance rather than stimulation of immune responses, because the effects of targeting known antigens can be modelled in mice and studied in human cell culture systems, enabling adverse effects to be detected. However, tumour-associated antigens are less likely than tumour-specific antigens to induce immune responses, as high-affinity T cells that respond to tumour-associated antigens are selected against during thymic T-cell development[37]. Compared with peptides derived from normal proteins that are overexpressed in gliomas, peptides from altered proteins that are only expressed in tumours, such as EGFRvIII or IDH^{R132H}, have the theoretical advantage of tumour specificity and a reduced risk of inducing autoimmunity. Few such antigens have been defined in glioblastoma. Thus, vaccination trials in patients with glioma, and the potential integration of vaccines into standard of care in this setting, might have to overcome obstacles not previously encountered in the clinical management of patients with brain tumours (FIGS 2,3).

Vaccines targeting EGFRvIII. The *EGFR* gene is amplified in approximately 40% of *IDH* wild-type glioblastomas[38,39]. More than half of such *EGFR*-amplified tumours exhibit a deletion mutation that results in expression of a truncated protein referred to as EGFRvIII[40]. This protein exhibits loss of the ligand-binding domain, resulting in constitutive kinase activity[41]. Moreover, the truncated protein exhibits a novel amino acid sequence, which has been identified as potentially immunogenic[31]. A vaccine known as rindopepimut employs this peptide sequence to evoke immune responses, and its efficacy against glioma was explored in three uncontrolled phase II clinical trials, which consistently demonstrated encouraging progression-free and overall survival data[42–44]. Admittedly, the patients vaccinated with rindopepimut in these trials were highly selected, in that the inclusion criteria required gross total tumour resection and an absence of progression at the first scan after completion of chemoradiotherapy[42–44]. The favourable results from these three trials provided the rationale for a pivotal phase III placebo-controlled trial of rindopepimut in patients with newly diagnosed glioblastoma (ACT-IV)[45]. Both groups of patients concurrently received standard maintenance temozolomide. However, an interim analysis conducted in early 2016 concluded that the ACT-IV trial should be terminated because the primary end point of improved overall survival was unlikely to be met[45].

Despite the negative outcome of this phase III trial, at least three important lessons can be learned from the results of ACT-IV: that the generation of strong humoral

immune responses to the vaccine did not translate into a survival benefit; that EGFRvIII expression was lost in approximately half of the patients in each arm of the study, indicating that EGFRvIII expression is not a stable feature of EGFR-amplified glioblastoma; and that a trend towards long-term survival benefit in vaccinated patients was detectable only in those with residual disease[45]. The latter observation contradicts the prevailing hypothesis that minimal residual disease (and, thus, absence of an extensive immunosuppressive micromilieu) is required for immunotherapy to be effective[45].

In parallel to ACT-IV, a smaller randomized clinical trial (ReACT) conducted in the USA compared rindopepimut with placebo in patients with recurrent glioblastoma[46]. Both groups of patients also received bevacizumab, the standard of care for this indication in the USA. Data are available in abstract form only, but overall survival seemed to favour the experimental arm (median 11.3 months; 95% CI 9.9–16.2 months) over the control arm (median 9.3 months; 95% CI 7.1–11.4 months; HR 0.53, $P = 0.0177$ in the per-protocol analysis[46]). The primary end point of improved progression-free survival at 6 months was not met, although a beneficial trend in the proportion of patients reaching this end point also favoured the experimental treatment: 28% of patients in the vaccine arm versus 16% of patients in the control arm ($P = 0.1163$) as ascertained by independent review[46].

Why vaccine therapy would show efficacy in patients with recurrent glioblastoma (who have been previously exposed to steroids, radiotherapy and temozolomide chemotherapy) while seeming not to improve outcome in those with newly diagnosed glioblastoma

(who should be relatively immunocompetent) remains challenging to understand. One might assume that previous exposure of the tumour cells to genotoxic stress exerted by radiotherapy and alkylating agent chemotherapy would make tumours more immunogenic; also, a transcriptomic profiling study indicated that recurrent tumours are more likely to exhibit a mesenchymal profile, which is characterized by expression of immune and inflammatory genes[47]. Furthermore, the patients in ReACT probably had more-extensive disease than those in ACT-IV, although we must assume, on the basis of data from ACT-IV[45], that only approximately half of the patients in the ReACT study would have had tumours that were still expressing EGFRvIII by the time of vaccination, as enrolment was based on EGFRvIII expression in the tissue obtained at initial surgery[46].

Although experimental data support the combination of vascular endothelial growth factor (VEGF) antagonism and immunotherapy[48,49], this combination might not be explored further as clinical development of bevacizumab in glioblastoma has been halted. Two trials reported no improvement in overall survival with bevacizumab therapy in newly diagnosed patients[37,38], and a further trial in patients with recurrent glioblastoma also reported negative results for bevacizumab treatment, albeit in combination with lomustine[50–52].

EGFRvIII has also been used as a target for chimeric antigen receptor (CAR)-based T-cell therapy[53], although as this strategy is not based on vaccination it is outside the scope of the present Review and will not be discussed further. Future trials will show whether CAR T-cell therapy exerts clinically meaningful antitumour activity in patients with glioblastoma.

Table 1 | Completed clinical trials of vaccination therapy for glioblastoma

Trial name and ClinicalTrials.gov identifier	Active treatment	Control	Sample size	Primary end point	Result
Phase III					
ACT-IV[135] NCT01480479	Rindopepimut plus GM-CSF	KLH plus GM-CSF	700	Overall survival	Negative
Phase II					
ReACT[136] NCT01498328	Rindopepimut plus bevacizumab	KLH and GM-CSF plus bevacizumab	70	Progression-free survival	Positive (trend)
HeatShock[137] NCT00905060	HSPPC-96 plus temozolomide	None	46	Safety and survival	Results pending
HSPPC-96 (REF. 138) NCT00293423	HSPPC-96	None	41	Safety, toxicity	Safe vaccine
GBM-Vax[139] NCT01213407	Trivax (a DC-based vaccine) plus temozolomide plus radiotherapy, followed by maintenance temozolomide	Temozolomide plus radiotherapy, followed by maintenance temozolomide	87	Progression-free survival	Results pending
Phase I					
IMA-950 (REF. 140) NCT01222221	IMA-950 plus GM-CSF	None	45	Safety and T cell responses	Positive for primary end point

Abbreviations: DC, dendritic cell; GM-CSF, granulocyte–macrophage colony-stimulating factor; HSPPC-96, heat-shock protein peptide complex 96; KLH, keyhole limpet haemocyanin.

Table 2 | **Ongoing clinical trials of vaccination therapy for glioma**

| Trial name and ClinicalTrials.gov identifier	Active treatment arms	Control	Sample size	Primary end point
Phase III				
DCVax[64] NCT00045968	DCVax	Autologous PBMC	348	Progression-free survival
STING[67] NCT02546102	ICT-107	Autologous monocyte-enriched PBMC	414	Overall survival
Phase II				
ATTAC-II[141] NCT02465268	Cytomegalovirus RNA-pulsed DCs plus tetanus–diphtheria toxoid	Unpulsed PBMC and saline	150	Overall survival
ALLIANCE IND#15380 (REF. 142) NCT01814813	HSPPC-96 and concomitant bevacizumab versus HSPPC-96 followed by bevacizumab at progression	Bevacizumab	165	Overall survival
HSPPC-96 (REF. 82) NCT03018288	TMZ–RT→TMZ plus pembrolizumab and HSPPC-96	• TMZ–RT→TMZ plus pembrolizumab and placebo • A separate group of patients whose tumours did not fulfil all inclusion criteria also received TMZ–RT→TMZ plus pembrolizumab and placebo	108	Overall survival at 1 year
SurVaxM[143] NCT02455557	SurVaxM	None	50	Progression-free survival
Phase I				
NOA-16 (REF. 76) NCT02454634	IDH^{R132H} peptide vaccine	None	39	Safety and tolerability
GAPVAC[34] NCT02149225	APVAC1 and APVAC2 vaccine plus poly-ICLC and GM-CSF	None	16	Safety and biological activity
NCT02287428 (REF. 59)	Personalized neoantigen vaccine	None	15	Feasibility and safety

APVAC, actively personalized vaccine; DC, dendritic cell; GM-CSF, granulocyte–macrophage colony-stimulating factor; IDH, isocitrate dehydrogenase; PBMC, peripheral blood mononuclear cells; poly-ICLC, polyinosinic polycytidylic acid (polyriboinosinic-polyribocytidylic acid); TMZ–RT→TMZ, radiotherapy with concomitant temozolomide followed by maintenance temozolomide; SurVaxM, a survivin-based 15-mer peptide with a Cys57Met amino acid substitution, conjugated to keyhole limpet haemocyanin.

Multipeptide vaccines. Multipeptide vaccines have gained increasing interest in the past few years. A vaccine consisting of three peptides derived from glioma-associated antigens has been explored in 26 HLA-A2-positive children with newly diagnosed diffuse intrinsic pontine glioma, anaplastic astrocytoma or glioblastoma[54]. Vaccination was safe and resulted in a measurable immune response[54], but no conclusions on efficacy can be derived from this uncontrolled study, which pooled several different disease entities. A similar vaccination strategy was also used in a phase I study of patients with WHO grade II glioma[55]. No dose-limiting toxicity was noted, and immune responses against at least three epitopes were observed in most patients[55].

Tumour-associated peptides derived from non-mutated proteins are shared to a high degree between different glioblastomas, and their presentation is thought to mainly reflect the presence of deregulated signalling pathways[56]. Patient-specific selection of tumour-associated peptides might enable the development of an individualized therapeutic cancer vaccine targeting antigens that are not abundantly present in the majority of glioblastomas, but show exceptionally high expression and potential immunogenicity in a given patient's tumour, thereby maximizing the chance of successful induction of relevant immune responses in that individual. IMA950 is one such multipeptide therapeutic vaccine developed for patients with glioblastoma. The IMA950 vaccine includes 11 tumour-associated peptides (nine HLA-A*02 class I peptides, an elongated class I peptide and one class II peptide), as well as the synthetic hepatitis B virus marker peptide IMA-HBV-001. A phase I trial of IMA950 in 45 patients with newly diagnosed glioblastoma receiving maintenance temozolomide has been completed. Treatment-related adverse events were generally mild, but two patients experienced dose-limiting fatigue or anaphylaxis[57]. Ultimately, 36 of 40 evaluable patients were characterized as single-peptide responders, and 20 patients were characterized as multipeptide responders (according to the vaccine-specific T-cell response criteria defined in the trial protocol). However, progression-free survival was only 74% at 6 months and 31% at 9 months, and median overall survival was 15.3 months[57].

Taking the concept of personalized vaccines to the next level of individualization, the Glioma Actively Personalized Vaccines Consortium (GAPVAC)
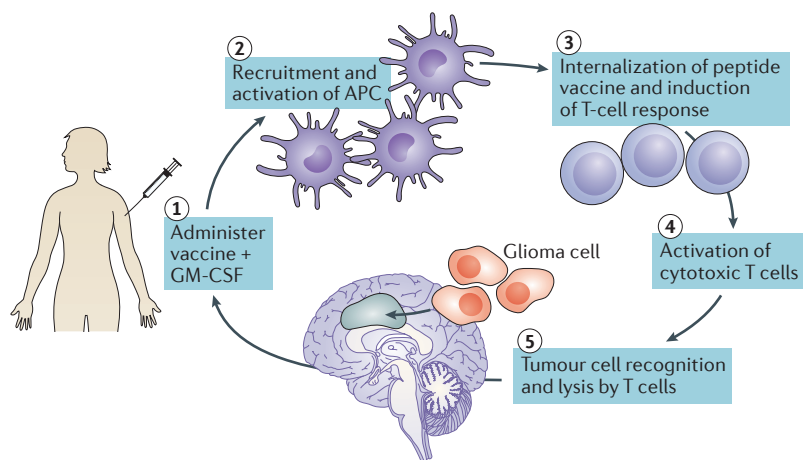
Figure 3 | **Putative mode of action for glioblastoma vaccines generated using tumour lysate or peptides.** Administration of the vaccine along with an appropriate adjuvant, such as granulocyte–macrophage colony-stimulating factor (GM-CSF), results in antigen-presenting cell (APC) activation and subsequent priming of T helper cells. These processes ultimately induce CD8+ T cell antitumour immune responses.

initiated a phase I trial of vaccines based on individualized selection of both tumour-associated peptides and tumour-specific peptides[34]. The term actively personalized vaccine (APVAC) had been previously coined by the Regulatory Research Group of the Association of Cancer Immunotherapy[58]. In the GAPVAC-101 trial, both APVACs were integrated into standard care (surgery and chemoradiotherapy followed by maintenance temozolomide) in newly diagnosed patients with glioblastoma[34]. APVAC 1 vaccines contained 5–10 peptides selected from a library of proteins obtained by expression profiling of the patient's tumour; the peptides most strongly associated with the tumour were selected for each patient to maximize the number of effective antitumour immune responses. APVAC 2 vaccines contained 1–2 custom-made synthetic mutated peptides. Next-generation sequencing and mass spectrometry were employed to compare the tumour and patient genomes and identify suitable mutated peptides for this purpose.

Another novel multipeptide vaccination strategy utilizes neoepitopes derived from mutant peptides expressed on an individual patient's tumour. A feasibility trial evaluating the administration of up to 20 neoepitopes per patient has been initiated as an individualized tumour vaccine strategy in patients with newly diagnosed glioblastoma[59]. Results of these approaches are eagerly awaited.

Dendritic-cell-based vaccines. DCs have been used for many years to generate vaccines for use in both paediatric and adult patients with glioma. Most clinical reports describe single-centre experiences, however, and many open questions remain with regard to the precise conduct of the treatment[60,61].

In an early phase I trial in adults with glioblastoma, autologous DCs were pulsed with acid-eluted autologous tumour peptides to yield the vaccine[62]. The researchers concluded that the absence of bulky, progressive disease and low expression of TGFβ2 defined a subgroup of

patients who might be most suitable for further studies of vaccine efficacy[62]. An ensuing phase I study in 23 such patients combined this vaccination approach with Toll-like receptor (TLR) agonist treatment[63]. The results led to the hypothesis that glioblastomas with a mesenchymal gene-expression profile exhibit increased immune cell infiltration associated with increased immunogenicity, and are, therefore, more amenable to immunotherapy than are tumours with other profiles[63]. DCVax is an ongoing phase III trial of a DC vaccine generated with autologous tumour lysate, and was based on the experience summarized above, but is not recruiting patients at present[64].

Another phase I–II trial assessed the activity of a DC-based multipeptide vaccine derived from glioma-associated antigens in 22 patients: 13 with glioblastoma, five with anaplastic astrocytoma, three with anaplastic oligodendroglioma, and one with anaplastic oligo-astrocytoma[65]. This trial yielded findings suggestive of clinical efficacy. In total, nine vaccinated patients (41%) — four with glioblastoma and five with anaplastic glioma — remained progression-free for ≥12 months[65]. The clinically most advanced DC-based vaccine to date is ICT-107, which is generated by exposing autologous patient-derived DCs to peptides derived from six proteins predicted to be abundant in glioblastoma and thought to be linked to the glioma stem cell signature[33,66]. These six proteins are glycoprotein 100 (gp100), melanoma-associated antigen 1 (MAGE1), interferon-inducible protein AIM2 (also known as absent in melanoma 2), tyrosine kinase-type cell surface receptor HER2 (also known as proto-oncogene Neu or receptor tyrosine-protein kinase erbB2), IL-13Rα2 (IL-13 receptor subunit α2), and tyrosinase related protein-2 (TRP2). The MAGE1 and AIM2 peptides were predicted to be HLA-A1-associated whereas the other four epitopes were predicted to be HLA-A2-associated[66]. The safety of the ICT-107 vaccine was confirmed in a phase I trial that enrolled 21 patients (17 with newly diagnosed glioblastoma, three with recurrent glioblastoma and one with brainstem glioma)[33]. Median progression-free survival in the newly diagnosed patients was 16.9 months, and median overall survival was 38.4 months, data which the researchers interpreted as encouraging. An increased duration of both overall survival and progression-free survival correlated with gene expression related to four of the six target proteins in the newly diagnosed glioblastoma cohort[33].

The ensuing randomized phase II trial of ICT-107 did not meet the primary end point of improved overall survival[66]. However, *post hoc* analyses revealed that a potential benefit was probably restricted to the subgroup of HLA-A2-positive individuals, who accounted for 77 of the 124 (62%) patients who underwent randomization. Moreover, the lack of an MRI scan to rule out progression at the time of randomization (that is, after completion of concomitant temozolomide and radiotherapy) might have accounted for the inclusion of patients with early progression in the group without promoter methylation of *MGMT* (methylated-DNA — protein-cysteine methyltransferase), which could have contributed to the failure to reach the primary end point[66]. On the basis of this

phase II experience, a pivotal phase III trial of ICT-107 has been initiated, which mandates confirmation of the absence of progression after completion of chemo-radiotherapy and limits enrolment to HLA-A2-positive patients[67].

IDH[R132H]-specific vaccines. Evidence for the therapeutic efficacy of IDH[R132H]-specific vaccines stems from pre-clinical studies in a humanized mouse sarcoma model[32] and an orthotopic syngeneic mouse glioma model[68]. *IDH1* is mutated in more than 70% of diffuse and ana-plastic gliomas, but only approximately 5% of glioblas-tomas[69]. The vast majority of *IDH1* mutations result in a protein with an arginine to histidine amino acid sub-stitution at position 132 (IDH[R132H])[70]. In addition to the metabolic and epigenetic consequences of this mutation with regard to gliomagenesis and tumour behaviour[71], IDH[R132H] harbours a neoepitope that, similarly to many other mutated antigens[72], is presented by professional antigen-presenting cells or MHC class II-expressing gli-oma cells[73], thereby stimulating mutation-specific CD4+ T-cell responses[74]. Indeed, spontaneous T-cell and anti-body responses to IDH[R132H] are observed in a fraction of patients with glioma[32]. The IDH[R132H] neoepitope also seems to be capable of presentation by multiple MHC class II allelotypes.

From an immunological perspective, the IDH[R132H] mutation represents an interesting target for immuno-therapy as it is not only tumour-specific but also expressed in all tumour cells and, thus, represents a clonal neoantigen with high uniformity and penetrance. In human immune responses to IDH[R132H], only muta-tion-specific CD4+ T cells have been observed[32]. In the absence of mutation-specific CD8+ T effector cells, the cellular mechanisms of the efferent arm of the thera-peutic response remain unclear, but the preclinical data suggest that B cells are required[75].

The ongoing NOA-16 trial[76] is a phase I safety, tolera-bility and immunogenicity multicentre study evaluating a 20-mer IDH[R132H] peptide in patients with treatment-naive WHO grade III–IV *IDH1*-mutated gliomas. Patient enrolment is not confined to a specific MHC class II haplotype, but the trial population is enriched for an unfavourable prognosis by restricting enrolment to patients whose tumours have an astrocytic molec-ular phenotype. Eight vaccines are integrated in the primary therapy, which in most patients comprises radio-chemotherapy combined with temozolomide. The trial is accompanied by a translational research programme that aims to identify key biomarkers for predicting and moni-toring response to the vaccine[76]. This important initiative will characterize the immunological mechanism of the (primarily T-helper-cell-driven) antitumour immune response, and promote the development of rational com-bination therapies. However, concerns regarding efforts to target mutant IDH[R132H] have been expressed, as this mutated protein negatively regulates the growth of glio-mas when exogenously transduced[77]. However, given that a vaccine would target cells expressing IDH[R132H] rather than the mutant protein itself, these concerns are unlikely to reflect the scenario of the therapeutic approach.

HSPPC-96. Heat-shock proteins (HSPs) are involved in cellular responses to stressors such as heat, from which this protein family derived its name. Notably, HSP-96 can bind tumour-associated antigens, and HSP-96 — peptide complex (HSPPC-96) can be taken up by antigen-presenting cells, potentially triggering specific antitumour responses[78,79]. HSPPC-96 has, therefore, been used to generate vaccines that aim to boost antitumour immune responses[80].

HSPPC-96 vaccination of patients with recurrent glioblastoma resulted in specific immune responses in the blood as well at the tumour site[81]. A subsequent phase II trial enrolled 41 patients with recurrent glio-blastoma who had undergone complete resection of the tumour. Survival was 90.2% at 6 months and 29.3% at 12 months[35]. In the absence of a control group in this trial, however, no statement on vaccine efficacy can be made[35]. Current limitations on the use of HSPPC-96-based vac-cines include the necessity of prior tumour resection, as 7 g of tumour tissue is needed to prepare at least four 25 μg doses of vaccine. A randomized phase II trial of HSPPC-96 vaccination in patients with newly diagnosed glioblastoma is ongoing[82] (TABLE 2).

Cytomegalovirus proteins. Several groups have demon-strated that human cytomegalovirus (CMV) proteins are expressed in >90% of glioblastomas[83–85], although other researchers have failed to detect human CMV protein or DNA in glioblastoma samples[86,87]. Expression of CMV protein has not been detected in normal brain tissue sur-rounding virus-positive glioblastomas[83,84,88,89], suggesting that viral antigens could be subverted as tumour-specific targets. Subclinical reactivation of latent CMV infection is frequent in critically ill and immunocompromised patients[90], and DCs pulsed with CMV antigens are potent inducers of virus-specific immune responses[91–95].

A study published in 2014 demonstrated that CMV-specific T-cell immune responses can recognize and effectively kill autologous glioblastoma cells expressing the immunodominant pp65 viral antigen at endogenous levels[96], supporting the development of CMV-directed immunotherapy. Moreover, CMV-reactive T cells might recognize glioblastoma cells independently of CMV antigen expression[97]. In a small randomized pilot trial, patients who received CMV pp65-specific DCs in combination with vaccine-site preconditioning using tetanus–diphtheria toxoid showed better than predicted progression-free survival (median 10.8 months) and overall survival (median 18.5 months) from diagnosis[98].

Boosting immune responses to glioma

As summarized in FIGURE 1, multiple mechanisms (including cell-surface-based and paracrine path-ways) mediate glioma-associated immunosuppres-sion[49,99–107], which is likely to limit the efficacy of active immunotherapy in the absence of effective counter-measures. Accordingly, this situation offers a strong rationale for combining specific immunological tar-geting via a glioma-specific vaccine with necessarily nonspecific approaches to reduce local and systemic immunosuppression, as described below.
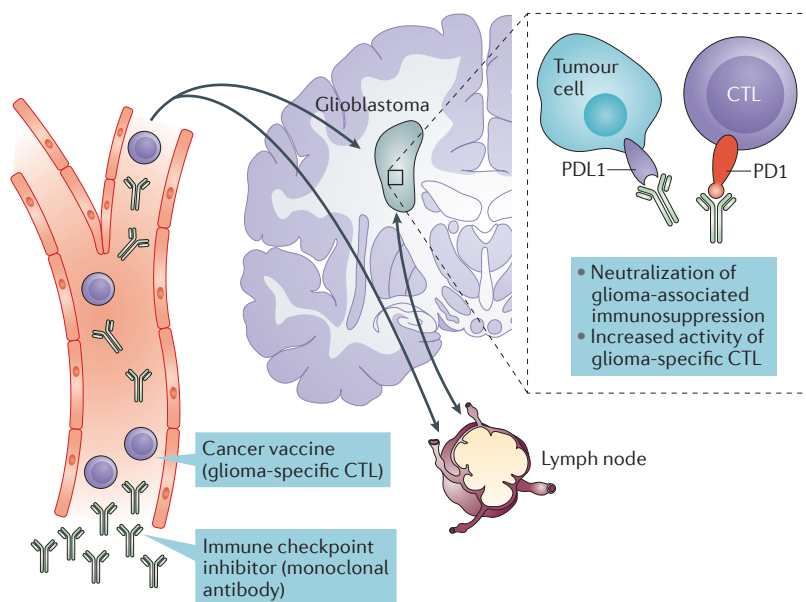
Figure 4 | **Facilitating effective vaccination by neutralizing glioma-associated immunosuppression.** Immune checkpoint inhibition using therapeutic monoclonal antibodies, such as those directed against PD1 (programmed cell death protein 1) or PDL1 (programmed cell death 1 ligand 1), might counteract glioma-associated immunosuppression in the tumour microenvironment or peripheral immune sites such as regional lymph nodes. Relief of tumour-associated immunosuppression could boost the activity of glioma-specific cytotoxic T lymphocytes (CTL) generated by vaccination.

Immune checkpoint modulators. The most straightforward approach to increase general immune responsiveness is administration of immune checkpoint modulators. Immune checkpoint inhibitors are therapeutic monoclonal antibodies that intercept receptor–ligand interactions involved in regulating immune cell activity. These drugs might, therefore, relieve glioma-associated immunosuppression and facilitate tumour cell destruction by immune cells[108] (FIG. 4). Immune checkpoint inhibitors such as ipilimumab (which targets CTLA4), nivolumab and pembrolizumab (which target PD1), and atezolizumab and durvalumab (which target PDL1) have shown remarkable and sometimes lasting activity against several cancer types, including melanoma, lung cancer, renal cell cancer, bladder cancer, head and neck cancer, and Hodgkin lymphoma[109–111].

High expression levels and immunosuppressive activity of the PD1–PDL1 axis have been documented in human glioblastoma[112–114]. Moreover, inhibition of this pathway results in substantial antitumour activity in rodent models of glioma[28,115], and PD1 inhibition in combination with DC vaccination prolonged the survival of glioma-bearing mice to a greater extent than treatment with either agent alone. Immune checkpoint inhibitors have also proven active against brain metastases in clinical trials[7], and a report published in 2016 documented clinically significant responses to the PD1 inhibitor nivolumab in two patients with recurrent glioblastoma who also had germline biallelic mismatch repair deficiency, providing proof of the concept that this therapeutic strategy is feasible in human CNS tumours[108,116].

Large clinical trials are currently evaluating the efficacy of immune checkpoint inhibitors in patients with newly diagnosed and recurrent glioblastoma[117].

Current preclinical research is not only evaluating additional immune checkpoint inhibitors, but also identifying checkpoint receptors that activate immune responses and so could be targeted using agonistic antibodies. Early preclinical data indicate that the co-stimulatory molecule OX40 (also known as CD134 or tumour necrosis factor receptor superfamily member 4) and its ligand OX40L (also known as CD252 or tumour necrosis factor ligand superfamily member 4) are involved in T-cell activation in glioblastoma[118]. Future research will show whether immune checkpoint modulators are capable of synergistically increasing the antitumour response of vaccination strategies against glioma in the clinical setting.

TGFβ inhibitors. Neutralizing the biological activity of TGFβ, the master immunosuppressive cytokine associated with glioma, also presents an option to improve the activity of vaccines for glioma treatment. The utility of this approach has been demonstrated in mouse models of glioma, in which treatment with TGFβ inhibitors enhanced the therapeutic efficacy of peptide vaccines and promoted CD70-mediated tumour rejection[27,119].

In humans, inhibition of TGFβ activity alone, using receptor tyrosine kinase inhibitors such as galunisertib, showed little activity in patients with glioma at first relapse[120]. Continuous dosing of such drugs is poorly tolerated, which necessitated a 2 weeks on, 2 weeks off dosing regimen in the clinical trial setting[120] that might be insufficient to inhibit the TGFβ–SMAD pathway to a relevant extent. Combinations of TGFβ inhibitors and a specific immunotherapy approach might still be worth exploring, however. For instance, the dosing of TGFβ inhibitors could be limited to specific weeks of active vaccine treatment.

Immunometabolic pathway inhibitors. The immunosuppressive microenvironment surrounding glioma is further maintained by metabolization of tryptophan, an essential amino acid. Tryptophan is cleaved by dioxygenases such as indoleamine-2,3-dioxygenase (IDO) or tryptophan-2,3-dioxygenase (TDO), resulting in several metabolites, but mainly kynurenine. Depletion of tryptophan and high levels of kynurenine both lead to impaired T-cell activation *in vitro*[121]. Hence, the addition of inhibitors of IDO, TDO or the aryl hydrocarbon receptor (AHR; a receptor for kynurenine that transduces its immunosuppressive signals to T cells and myeloid cells), could help to increase the activity of immune responses following vaccination[122,123]. Similarly, inhibition of the activity of signal transducer and activator of transcription 3 (STAT3), which is a central regulator of various glioma-derived immunosuppressive mechanisms, might represent a therapeutic strategy to overcome the immunosuppressive tumour microenvironment[124]. Such concepts are only at an early stage of clinical development; however, a phase I study in patients with recurrent malignant glioma or brain metastasis from melanoma is ongoing[125].

Targeting regulatory T cells. Patients with glioblastoma are profoundly immunosuppressed, at least partly as a result of an excessive number of immunosuppressive myeloid-derived suppressor cells (MDSC) and regulatory T cells (T_{reg} cells)[126–129]. Key features of this immunosuppressive phenotype can be reversed by eliminating T_{reg} cells[126]. When T_{reg} cells are depleted *in vitro*, T-cell proliferation and cytokine secretion return to normal levels[126]; *in vivo* depletion of T_{reg} cells 1 week after tumour implantation prolonged the survival of mice inoculated with glioma cells, demonstrating not only that T_{reg}-mediated immunosuppression is reversible, but also that T_{reg} impairment positively influences antitumour immunity[130].

Previous attempts to eliminate T_{reg} cells have had mixed outcomes[131,132], possibly because T_{reg} depletion was not employed in the unique host environment that exists after therapeutic temozolomide-induced lymphodepletion. These strategies might also have failed because they employed the IL-2 moiety itself to target T_{reg} cells. This method would result in indiscriminate targeting of low-affinity IL-2βγ receptors, which are expressed on a broad subset of immune cells, including memory T cells. Alternative strategies that employ IL-2Rα-targeted immunotoxins result in rapid and indiscriminate killing of all IL-2Rα-expressing cells, which might include recently activated, vaccine-induced effector T cells. In marked contrast, IL-2Rα-specific antibodies lacking the bound immunotoxin eliminate T_{reg} cells while having no effect on effector T cells in lymphopenic mice[133].

Conclusions and future perspectives

Glioblastoma remains one of the most-studied tumours in the context of cancer-associated immunosuppression. Numerous soluble mediators and cell-based pathways of immunosuppression were first delineated in glioblastoma, and these observations collectively support further efforts at establishing efficacious antitumour immunotherapies. Although proof of efficacy is not yet available for any of the glioma-specific peptide vaccines currently in clinical development, the addition of immune checkpoint inhibitors or other approaches that boost immune responses in vaccinated patients might ultimately be able to demonstrate that active immunotherapy can control the growth of human intracranial neoplasms.

Meanwhile, the clinical development of immunotherapy for glioblastoma could be aided by collective efforts to introduce measures of quality control and standardization of inclusion and exclusion criteria, as well as standardized response and other efficacy criteria in immunotherapy trials. The iRANO (Immunotherapy Response Assessment in Neuro-Oncology) criteria, which essentially caution against the premature assumption of inefficacy or treatment failure in early phase clinical trials of immunotherapy[134], are just a first step in this direction. The identification of tumour or serum biomarkers that predict response or progression of glioblastoma is needed to improve the conduct and efficiency of clinical trials. Such efforts will require international collaboration involving all the major organizations and disciplines involved in the orchestration of care for patients with brain tumours.

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