Maximizing output from current glioma vaccine trials to construct robust next-generation immunotherapies

"Immunotherapy for brain tumors is entering an exciting era with real opportunities for clinical impact using safe, broadly applicable therapeutic vaccines that are already under clinical trial."

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High-grade gliomas are highly heterogeneous malignant tumors with poor prognosis, accounting for approximately 40% of primary CNS tumors. The absence of major progress with conventional treatment modalities (i.e., surgery, radiotherapy and chemotherapy) and the proven chemo- and radio-resistance of glioma cells have encouraged research into immunotherapy. This is supported by developing concepts in tumor immunology and by rational immunotherapies for certain malignancies that are starting to have clinical impact [1]. However, for brain tumor immunology to progress towards brain tumor immunotherapy, it has first been necessary to debunk a number of myths and scientifically establish that:

- Immune responses do occur in the immune-privileged brain;
- Brain tumors are antigenically distinct from their normal cellular counterparts;
- The blood–brain barrier does not totally block brain entry of therapeutic lymphocytes;
- Most critically, brain tumors can be rejected by immune mechanisms without overt toxicity [2].

The major challenge now is how best to develop and optimize brain tumor immunotherapies to achieve clinical impact for patients with glioma. The immune system (either 'natural' or 'engineered') offers many options for therapeutic application, including promising adoptive cell transfer therapies (T cells and dendritic cells [DCs]) [3]. Certain approaches are technologically complex and will be clinically tested on a small scale in the foreseeable future. In parallel, we propose that a fast-track, complementary approach to improve glioma immunotherapies will be achieved through optimizing standardized, defined vaccines, particularly through vaccine formulation and adjuvant selection. Indeed, basic and clinical data should be exploited to guide us towards higher efficacy vaccines testable in the near future.

From a basic immunology standpoint, the *sine qua non* for T cell-based immunotherapy is targeting a tumor-expressed antigen. And yet we frequently attempt the impossible – we try to judge glioma vaccine efficacy after inducing immune responses to unknown antigens (e.g., those presumed to be present in tumor lysate or complexed with heat shock proteins) or by using defined peptide vaccines, but with nondefined expression by the tumor cells of each vaccinated individual [2,4]. Nevertheless, there are now major advances in defining glioma antigens expressed *in vivo* [5]. Moreover, there are now encouraging clinical responses to suggest that immunotherapy is sometimes 'on target', although not for the majority of patients. In the absence of 'perfect' glioma-specific antigens uniformly expressed by the tumor, there are no easy solutions for vaccine design and interpretation; we should therefore be cautious about rejecting a given vaccine formulation based on negative clinical results. The way forward will require continued use of animal models, in which much maligned model tumor antigens still play an important role when investigating vaccine potency, or the role of T-cell subsets in antiglioma immunotherapies [6].

Correlating immunological and clinical response data is a burning issue that clouds interpretation of most tumor immunotherapy studies, but the challenge is particularly demanding for gliomas because of tumor site inaccessibility. A peripherally administered vaccine will induce an antigen-specific immune response that is detectable at some stage at some site. In clinical

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situations immunomonitoring is most feasible in blood, but in animal models, any site is accessible (lymphoid tissue and tumor bed). In both situations, sensitive, reproducible cellular assays are required, but for which there is currently no 'gold standard'. This has been recognized and addressed by the Minimal Information about T cell Assays (MIATA) project, which provides a framework to report and interpret T-cell immunomonitoring data [7]. This developing initiative provides the prospect of better comparability of future vaccination trial data. However, it remains to be determined whether monitoring tumor-specific T cells in blood will correlate with T-cell infiltration of the tumor bed, and with clinical impact. In this regard, we demonstrated discordant results for the presence of tumor-specific CD8 T cells in blood and at the brain tumor site in mouse models [8]. In humans, recent DC-based glioma immunotherapy validated that tumor-infiltrating antigen-specific T cells were correlated with clinical impact [9]. Such cells are mandatory, but not necessarily sufficient for antitumor function, and exploration of T-cell effector function and persistence should continue to be a major research objective despite inherent site-related challenges.

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In the absence of T-cell monitoring at the tumor site of patients, are there predictive markers for T-cell tropism for brain tumor? When T cells are primed in lymph nodes they are imprinted with a 'homing phenotype' (adhesion molecules and chemokine receptors) that influences migration to different sites, with VLA-4 ($\alpha_4\beta_1$ integrin) and CXCR3 expression by CD4 and CD8 T cells facilitating brain tumor infiltration [6,10,11]. Of particular relevance for therapeutic immunity, expression of these key receptors is associated with type 1 polarization, critical for antitumor function. Thus, we propose that in addition to immunomonitoring for antigen-specific immune responses, we should also assess T-cell homing molecule expression to validate correlation with tumor infiltration in patients, and ultimate clinical outcome.

Based on the preclinically validated hypothesis that targeting valid glioma antigens with type 1-polarized VLA-4$^+$/CXCR3$^+$ T cells impacts on tumor growth, how can we best achieve a high magnitude response of this type by vaccination? Immunology has taught us that antigen-specific T cells are stimulated and imprinted with these desirable properties by APCs. Of these, DCs are the most potent and have multiple phenotypes, functions and capacities to stimulate T cells; we can induce appropriate DC function by use of appropriate immunologic adjuvants. Although numerous adjuvants have been tested in preclinical models, some have little interest for therapeutic cancer vaccines (e.g., alum), and only incomplete Freund's adjuvant (IFA)/montanide, GM-CSF, CpG, polyinosine–polycytidylic acid (poly-IC) and imiquimod have undergone significant (although not comparative) testing in the context of glioma vaccines.

IFA/montanide

These oil and water emulsion-based adjuvants are widely used experimentally in preclinical and clinical glioma studies. Their perceived interest is their depot effect allowing sustained antigen release. However, rigorous testing of peptide vaccines ± IFA (in mice) provided compelling evidence that vaccine-induced CD8 T cells can be sequestered and inactivated at the vaccination site, which was most pronounced for formulations using minimal peptide epitopes [12]. Unless clinical data validates a positive role for IFA/montanide, this adjuvant would appear to have little future for therapeutic cancer vaccination.

GM-CSF

There is a wealth of preclinical and clinical data for this cytokine, which when used as a vaccine adjuvant can recruit DCs to the vaccination site and enhance antigen presentation. It is well-tolerated and has shown positive effects in the context of past and ongoing glioma vaccination trials [13]. However, induction of suppressive cells with certain doses (generally higher) and administration schedules [14] can jeopardize antiglioma immune function.

CpG

The Toll-like receptor (TLR)-9 agonist CpG has shown promise as a vaccine adjuvant for certain tumors and has also been explored in glioma with encouraging data in mouse models, although it was not compared with other adjuvants [15]. However, the most advanced studies with CpG were not as a vaccine adjuvant, but by intratumoral delivery as a monotherapy, with positive preclinical data [16], but only minor effects (not meeting targeted progression-free survival)

in a Phase II clinical trial in recurrent glioma [17]. Absence of detailed immunomonitoring or tumor analysis after treatment makes interpretation difficult, but the immunogenicity of mouse versus human glioma may be an important issue for this approach without vaccination.

Imiquimod

The TLR-7 agonist imiquimod has been mainly explored for topical application, this being the route licensed for clinical use. As a monotherapy in murine glioma, imiquimod enhanced tumor infiltration by effector T cells and DCs, while reducing Tregs [18]. In a DC-based glioma vaccine clinical trial, topical imiquimod (and also poly-IC) was safely used as an adjuvant, although the importance of the adjuvant in the study was not assessed [9].

Poly-IC

The TLR-3 and MDA-5 agonist poly-IC (and its stabilized form poly-ICLC-Hiltonol®) is a much-explored vaccine adjuvant with particularly interesting properties for glioma. In addition to the direct DC-maturing, immune-enhancing adjuvant effects expected from most TLR agonists, there is abundant CNS expression of TLR-3 that may promote favorable modulation of the glioma microenvironment through stimulation of proinflammatory cytokine and chemokine release. Moreover, poly-IC adjuvanted peptide or DC-based vaccines induced glioma-specific T cells expressing homing receptors appropriate for efficient brain tumor homing [19]. These and other data have encouraged use of poly-IC in over 20 clinical trials in glioma, with promising clinical and immunological data [20].

Immunotherapy for brain tumors is entering an exciting era with real opportunities for clinical impact using safe, broadly applicable therapeutic vaccines that are already under clinical trial. However, the opportunities now available for cancer immunotherapy exceed what can be rationally tested clinically in glioma. Vaccination combined with immunologic checkpoint blockade, or with nonimmunologic treatment modalities, or use of engineered effector cells, offer the prospect for even greater efficacy. Fortunately, some of these approaches will be refined (and toxicity minimized) in other cancer indications before being available for glioma. In the meantime, this delay before testing combination glioma therapies offers an opportunity to incorporate the maximum information from optimized vaccine trials with robust immunomonitoring, as well as integrating data from more sophisticated animal models, and use of a wider palette of glioma antigens for both CD4 and CD8 T cells.

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