Cytomegalovirus as a novel target for immunotherapy of glioblastoma multiforme

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INTRODUCTION

Temozolomide was the last drug to bring a significant improvement of survival for patients with glioblastoma multiforme (GBM) brain cancer (1). Now, almost a decade later, further major advances in drug development have remained elusive. GBM continues to be the most aggressive human brain cancer with 5 year survival rates below 10% (2). Standard of care treatment includes surgical resection followed by radiation and chemotherapy, however, even with optimal treatment median survival is only 15 months (1). Glioblastomas are incurable and inevitably recur with a median survival of only 6 months (3). There is an urgent need to find novel therapeutic targets and develop new treatment strategies. Immune-based approaches have great potential, in particular, because they have the great advantage of being safer and less toxic than chemotherapy drugs (4). Bevacizumab (brand name Avastin) is the only immunotherapy drug currently approved for the treatment of recurrent GBM. Bevacizumab is a monoclonal antibody that blocks vascular endothelial growth factor (VEGF), thereby reducing angiogenesis. Recently, two large clinical studies evaluating bevacizumab for the treatment of primary GBM indicated a prolonged progression free survival but failed to show a significant benefit for overall survival (5, 6). Glioblastomas are very heterogeneous tumors and it is likely that a single agent will be insufficient to achieve therapeutic benefit in a majority of patients. More than 30 studies are currently underway to assess the potential of combination therapies using bevacizumab together with radiation or other chemotherapy drugs (7). Most cellular immunotherapies for GBM under investigation at the moment focus on using tumor lysate loaded dendritic cells (DCs) or vaccination using tumor peptides [for an overview see recent review in Ref. (4)]. Preliminary results have shown some promise, however, further optimization is needed to improve anti-tumor immune responses.

CYTOMEGALOVIRUS AS A TARGET FOR GBM IMMUNOTHERAPY

The first report of detection of cytomegalovirus (CMV) antigens in histological sections of GBM (8) was received with much controversy. Subsequent studies have disputed (9–11) as well as confirmed (12–14) the original finding. While CMV sequences have been detected in GBM tissues (15, 16), the topic continues to be controversial as recent deep sequencing studies fail to detect CMV in GBM (17, 18). Interestingly, a clinical study evaluating vaccination using DCs pulsed with autologous tumor lysate described a patient that developed enhanced CMV-specific T cell responses after one dose of the vaccine. No such response was found in a patient with a CMV negative tumor enrolled into the same study, which provided the first immunological evidence for the presence of CMV antigens in glioma cells (19). Specific killing of primary GBM cells by autologous CMV-specific T cells was demonstrated recently and further argues for the existence of CMV antigens in brain tumors (20). The precise role of CMV in GBM remains unclear. In contrast to other members of the herpes virus family like Epstein–Barr virus, CMV is not an oncogenic virus. However, CMV encodes many genes that may enable hallmarks of cancer such as pro-angiogenic signaling, immune evasion, and deregulation of the cell cycle (21–26). From a therapeutic perspective, the presence of CMV antigens provides a unique opportunity to exploit pre-existing antiviral immunity for immune-based GBM treatment. Further support for this rationale stems from the finding that lower levels of CMV antigens in GBM sections are associated with prolonged
survival (27, 28). Therapeutic use of antiviral drug valganciclovir for GBM patients has been explored without major side effects, but further studies are needed to assess efficacy (29–31). In the context of immunotherapy, a previous study of CMV-specific T cell responses in GBM patients has detected functional impairment of antiviral T cells. Despite similar frequencies in GBM patients and healthy individuals, CMV-specific cytotoxic T cells in GBM patients showed limited ability to produce multiple cytokines (macrophage inflammatory protein MIP-1β, tumor necrosis factor TNF, interferon IFNγ) and to mobilize CD107a in response to CMV epitopes (32). Importantly, polyfunctionality of CMV-specific T cells isolated from GBM patients could be restored by in vitro stimulation with CMV antigens and γC cytokines (32). This suggests that adoptive transfer of in vitro expanded T cells could improve CMV-specific immune responses in GBM patients. Indeed, this preliminary study has shown that immunotherapy using CMV-specific T cells was coincident with prolonged survival in one patient (32). CMV-specific immunity is characterized by high frequencies of CMV-specific cytotoxic T cells in seropositive individuals. While tumor associated antigens are usually poorly immunogenic, viral antigens provide a strong stimulus that makes expansion of high-frequency antiviral T cell cultures comparatively easy. Consequently, several laboratories have established efficient protocols for the in vitro expansion of CMV-specific cytotoxic T cells from GBM patients for the purpose of immunotherapy (20, 32, 33). Efficient killing of autologous primary tumor cells by CMV-specific T cells was demonstrated in vitro, thus, providing direct evidence that CMV-specific cytotoxic T cells can be applied for GBM therapy (20).

CURRENT INVESTIGATIONS OF CYTOMEGALOVIRUS SPECIFIC GBM IMMUNOTHERAPY

Two clinical studies using CMV-specific immunotherapy are currently recruiting participants and two further studies using CMV-specific autologous lymphocyte transfer and DC vaccination have completed enrollment (Table 1). Of the currently recruiting studies, one study is testing genetically modified CMV-specific cytotoxic T cells for the treatment of recurrent GBM (clinical trials identifier NCT01109095). CMV-specific T cells are engineered to express a chimeric antigen receptor (CAR) recognizing human epidermal growth factor receptor 2 (HER-2) coupled to CD28 (7). HER-2 antigen is expressed on a majority of GBM cells and CD28 promotes sustained T cell activity. While the primary objective of the study is safety, it might provide some insight if the targeting of glioma cells using T cells specific for CMV and HER-2 can provide a survival benefit. The second CMV-specific GBM immunotherapy under investigation is based on a combination of DC vaccination and a monoclonal antibody directed against CD25, which blocks interleukin-2 signaling (clinical trials identifier NCT00626483). Autologous DCs are loaded with CMV pp65-LAMP mRNA and administered with different doses of CD25 antibody, which is expected to inhibit regulatory T cells (7, 34). In this approach, alleviating the suppressive effect of regulatory T cells might lead to more efficient CMV-specific T cell activity. Results from these studies are expected by 2016 and 2015, respectively.

FIRST CLINICAL OUTCOMES OF CYTOMEGALOVIRUS SPECIFIC THERAPY FOR GBM

The first formal clinical assessment of CMV-specific adoptive T cell immunotherapy was completed recently (35). Ten CMV seropositive patients with recurrent GBM received three or four infusions of autologous CMV-specific cytotoxic T cells generated following in vitro stimulation with synthetic CMV epitopes. The treatment was shown to be safe and only mild side effects were recorded. While the patient cohort was too small to draw definite conclusions about efficacy and effects on overall survival, it is notable that 4 out of 10 patients remained completely disease-free during the study period. At present, the follow up time for these patients since initiation of T cell therapy ranges from 10 months to more than 4 years, which extends well beyond the expected survival median time of 6 months after tumor recurrence. Immunological and molecular analysis revealed a number of important insights. Analysis of a range of immunological parameters on peripheral blood mononuclear cells (PBMC) before and after T cell therapy failed to reveal major changes. In contrast, molecular analysis of the T cell product used for adoptive therapy showed a signature of seven genes (EOMES, IFNG, BCL6, XAF1, CCL5, CTLA-4, FOXP3) that distinguished individuals with long-term progression free survival from patients that progressed more rapidly. This signature was consistent with T cell activation (i.e., upregulation of T cell transcription factor Eomes and effector molecule IFNγ, downregulation of inhibitory receptor CTLA-4) suggesting that more functional CMV-specific T cells are more efficient in controlling cancer relapse. One patient had a tumor recurrence after therapy and isolation of T cells

Table 1 | Current clinical trials evaluating CMV-specific immunotherapy for GBM.

| Intervention | GBM type | Enrollment | Phase | Duration | NCT number | Status |
|--------------|----------|------------|-------|----------|------------|--------|
| Genetically modified HER.CAR CMV-specific CTLs | Recurrent | 18 | I | 2010–2031 | NCT01109095 | Recruiting |
| DC vaccine (CMV pp65-LAMP mRNA loaded DC), basiliximab (anti-CD25) | Primary | 18 | I | 2007–2015 | NCT00626483 | Recruiting |
| DC vaccine (CMV pp65-LAMP mRNA loaded DC) with or without autologous lymphocyte transfer, tetanus toxoid | Primary | 16 | I | 2006–2016 | NCT00639639 | Active, not recruiting |
| CMV autologous lymphocyte transfer with or without DC vaccine (CMV pp65-LAMP mRNA loaded DC) | Primary | 12 | I | 2008–2016 | NCT00693095 | Active, not recruiting |
from the resected tissue revealed the first evidence that CMV-specific T cells are present in tumor tissues. In addition, intratumoral T cells were found to express higher levels of immune inhibitory molecules, indicating that local immunosuppression might be an important factor in the development of effective therapies.

**IMMUNE REGULATORY MECHANISMS TO CONSIDER**
Immunosuppression is one of the classic hallmarks of human cancers (36). In the context of GBM, this principle applies locally in the tumor microenvironment as well as systemically (4). Immune inhibitory receptors expressed on T cells such as PD-1, CTLA-4, TIM-3, or BTLA play an important role for modulation of T cell responses. Expression of PD-L1, the ligand for PD-1, has been detected in glioma samples (37) and a recent study found high-PD-L1 levels in tumor tissue to be associated with poor survival (38). Blockade of such checkpoint inhibitors might therefore be an attractive treatment option to boost intrinsic tumor defenses. Interference with the PD-1/PD-L1 interaction by monoclonal antibodies directed against PD-1 is currently under clinical investigation for the treatment of recurrent GBM (clinical trials identifier NCT01952769). Similarly, a monoclonal antibody blocking CTLA-4 (ipilimumab) that is currently approved for the treatment of metastatic melanoma might be effective in enhancing anti-glioma immune responses. A combination therapy of anti-VEGF and anti-PD-1 or anti-CTLA-4 is in phase II testing for recurrent GBM (clinical trials identifier NCT02017717). In the context of CMV-specific immunotherapy for GBM, analysis of intratumoral CMV-specific T cells in a patient that relapsed after T cell therapy indicated that local immunosuppression might have contributed to treatment failure (35). While further testing is needed to confirm this finding in a larger patient cohort, it is tempting to speculate that combination of CMV-specific T cells with blockade of inhibitory receptors might boost efficacy of adoptive immunotherapy.

**CONCLUSION AND PERSPECTIVE**
Immunotherapies provide a novel approach to complement standard therapies for GBM treatment (Figure 1). CMV antigens provide an attractive target for cellular immunotherapies that
could provide more efficient tumor recognition than tumor associated antigens. The first clinical assessment of CMV-specific T cell therapy has been completed and proved to be safe with potential clinical benefit. Further clinical trials using CMV directed immunotherapy for GBM are underway. Cancer associated immunosuppression has to be taken into account as it might limit the effectiveness of antiviral T cells within the tumor tissue. Future studies should therefore focus on multimodal strategies combining cellular immunotherapy with blockade of inhibitory receptors on T cells.

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