Immune infiltration of tumor microenvironment following immunotherapy for glioblastoma multiforme

Mr Giannis Sokratous, Dr Stavros Polyzoidis & Keyoumars Ashkan

To cite this article: Mr Giannis Sokratous, Dr Stavros Polyzoidis & Keyoumars Ashkan (2017): Immune infiltration of tumor microenvironment following immunotherapy for glioblastoma multiforme, Human Vaccines & Immunotherapeutics, DOI: 10.1080/21645515.2017.1303582

To link to this article: http://dx.doi.org/10.1080/21645515.2017.1303582

Accepted author version posted online: 31 Mar 2017.

Article views: 55

View related articles

View Crossmark data
Immune infiltration of tumor microenvironment following immunotherapy for glioblastoma multiforme

Mr Giannis Sokratous, MSc, MRCS\textsuperscript{1}, Dr Stavros Polyzoidis, MD, PhD\textsuperscript{2}, Professor Keyoumars Ashkan, BA, BSc, MB, BCh, MRCP, FRCS, FRCR, FRCPS, FRCS (SN), MD, Professor of Neurosurgery\textsuperscript{3}

\textsuperscript{1}Clinical Research Fellow, Department of Neurosurgery, King’s College Hospital, Denmark Hill, SE5 9RS

\textsuperscript{2}Clinical Fellow, Department of Neurosurgery, King’s College Hospital, Denmark Hill, SE5 9RS

\textsuperscript{3}Department of Neurosurgery, King’s College Hospital, Denmark Hill, SE5 9RS

Email: giannis.sokratous@nhs.net

**Disclosure of potential conflict of interests and financial disclosure.** No potential conflicts of interest or financial benefits to disclose.

**Abstract**

**Background:** Autologous dendritic cell immunotherapy has been proven effective in treating tumors outside the central nervous system. Current evidence from phase I and II trials suggest a similar efficacy for central nervous system tumors as well and that an active immune response against these tumors can be generated. **Objective:** We aim to review the literature to identify the types of immune responses against gliomas found to be generated by dendritic cell vaccinations and the types of immune cells subsequently infiltrating the glioma microenvironment. **Methods:** A systematic review of the literature was performed by searching the online databases PubMed, Google Scholar, and EMBASE with use of the keywords intratumoral, infiltration, lymphocytic, vaccination and gliomas. **Results:** Seven studies reporting lymphocytic infiltration of gliomas microenvironment were identified. Three studies (42.8\%) reported presence of tumor infiltrating lymphocytes in 50\%, 50\% and 28.6\% of included patients respectively in the post–vaccination specimens that were not present in the pre–vaccination samples. The remaining 4 (57.2\%) reported an up to six-fold increase in the number of pre-existing lymphocytes following vaccination. **Conclusion:** Present data indicate that tumor infiltration by lymphocytes can be induced by dendritic cell immunotherapy and that this may positively affect clinical outcome. It still remains unclear which factors influence the above reaction and therefore prediction of response to treatment is still not possible.

**Keywords**

Intratumoral, infiltration, lymphocytic, vaccination, glioma
Introduction

Background

Glioblastoma multiforme (GBM) remains one of the most lethal tumors despite recent treatment advances in neuro-oncology. Treatment with combination of surgical resection, radiotherapy and chemotherapy is standard of care and provides a statistically significant survival benefit with minimal additional toxicity when compared to treatment with resection and radiotherapy alone. Despite the latter though, overall survival remains very poor with median overall survival of 14.6 months, 2 year survival of 26.5% and 5 year survival of 9.7%. Different approaches are currently being investigated with immunotherapy being one of the most appealing and currently under intense investigation. Immunotherapy aims at boosting the patients’ immune system to recognize and attack tumor cells.

The infiltration of both animal and human tumors by lymphocytes is known for more than a century now and in the 1960s a possible relation between infiltration and prognosis was considered. Hamlin was the first to show the relation between lymphocytic infiltration and prognosis in patients with breast cancer. Other studies showed that the majority of cells infiltrating tumors were T cells (80%) and that there was positive correlation between the degree of lymphocytic infiltration of primary tumors and the absence of metastases. Bertrand and Mannen, in 1960, were the first to report infiltration of glioma microenvironment by lymphocytes, followed by Lucio Palma who showed that glioblastoma patients with definite lymphocytic infiltration had significantly longer postoperative survival.

The presence of the blood-brain barrier, the unique lymphatic drainage of the central nervous system (CNS), as well as the distinct and localized actions of the microglia create an immunologically challenging environment within which brain tumors grow, escaping regular immune surveillance observed in peripheral tissues. Additionally, it has been shown that in patients with GBMs, there is under-expression of immunostimulatory MHC class I molecules and overexpression of suppressive surface proteins (PD-L1, FasL) and cytokines (IL-10, TGF-b, CCL) which stimulate the accumulation of T regulatory cells and myeloid derived suppressor cells. This leads to impaired proliferation and activation of cytotoxic lymphocytes. Thus accumulation of natural killer and regulatory T cells causes leukopenia and immunological compromise (Figure 1).

Evidence that the “immunologically privileged” environment of the central nervous system can be overcome and that an immune response against brain tumors can be generated....
by vaccination with cytokine–producing tumor cells was first described almost twenty years ago. In this study it was shown that tumor–bearing mice had increased overall survival (OS) following subcutaneous vaccination with genetically engineered tumor cells. Interestingly, in the same study, they concluded that, unlike tumors outside the CNS, where the presence of both CD4 and CD8 cells is necessary for an effective response, relative depletion of CD4 cells with increased CD8 cells is associated with better outcome with CNS tumors\(^\text{13}\). A significant number of other preclinical studies on various grades of tumors showed similar results with reports of an increase in the OS of animals injected with tumor cells previously cultured with antigen presenting cells\(^\text{14,15}\). Notably, even complete resolution was reported in some cases\(^\text{16}\).

The above observations, combined with the need for a treatment that is both patient and tumor specific as well as safe, triggered research efforts on passive and active immunotherapy for brain tumors. The latter is almost exclusively based on dendritic cell (DC). Of significance, peripheral vaccination with DCs pulsed with tumor antigens has already shown positive results in other types of tumors, including prostate, melanoma, lymphoma and renal cell carcinoma, with minimal side effects and no observed autoimmunity, rendering the technique safe\(^\text{17-20}\).

Dendritic cells are the most powerful of antigen presenting cells and are able to activate both naïve and memory immune responses. In dendritic cell based immunotherapy for tumors, immature cells are isolated from the patients via leukapheresis. Addition of proinflammatory cytokines induces their maturation which is followed by loading with tumor antigens\(^\text{21}\). Once the complex is mature, it is injected back to the patient where dendritic cells act to present the antigens to CD8 and CD4 T cells via MHC class I and II, inducing tumor specific response\(^\text{22}\) (Figure 1).

**Objectives**

In this study we aim to review the literature and identify all studies reporting lymphocytic infiltration of the tumor microenvironment following peripheral (subcutaneous or intradermal) vaccination with antigen pulsed DCs for the treatment of intracranial high-grade gliomas (HGGs). Additionally we aim to present the evidence relating to the importance of lymphocytic infiltration in human gliomas.

**Methods**

3
Inclusion criteria

- Phase I/II or prospective studies evaluating the efficacy of adjuvant vaccination using DCs previously pulsed with tumor antigens in the treatment of HGGs managed with standard of care protocols.
- Human patients
- Studies reporting intracranial / intratumoral lymphocytic infiltration
- Published in English

Literature review

Using the keywords *intratumoral (intratum$)*, *infiltration (infiltr$)*, *lymphocytic (lymphocyte$)*, *vaccination (vaccin$)* and *gliomas (gliom$)* a systematic review of the literature was performed by searching the online databases PubMed, Google Scholar, and EMBASE. To ensure no studies were missed, the references of included studies were also reviewed. Last search of the literature was performed on 08 November 2015.

Data collection

- General data
  - Author, country, number of patients, trial phase, mean age, I.D or S.C vaccination
- Treatment protocol
- Intratumoral infiltration
  - Number of patients, means of identification
- Outcome

Results

Studies

Original search revealed 29 studies. Removing duplicates left 20 studies for full review. Following further removal of studies not commenting on intracranial or intratumoral infiltration, animal and non – English studies left 7 that fulfilled all inclusion criteria. Main characteristics of the included studies are presented in Table 1.

General Characteristics of included studies

Three of the above studies took place in the U.S.A (42.8%), one in Taiwan (14.2%), one in Germany (14.2%), one in Australia (14.2%) and one in Japan (14.2%). Four studies
were phase I (57.1%), two were phase I/II (28.5%) and one prospective study (14.2%). The number of patients included varied from 12\textsuperscript{22} to 24\textsuperscript{23} with the mean age varying from 44.7\textsuperscript{24,25} to 51\textsuperscript{26,27}. The vaccination was administered intradermally (ID) in 5 out of 7 studies (71.4%), subcutaneously (SC) in 1 (14.2%) and either intradermally (ID) or intrathecally (IT) in one (14.2%).

In 6 out of 7 studies the vaccine was produced with tumor antigens pulsed with dendritic cells (DC) that were harvested from the patients’ peripheral circulation (85.7%), whilst in 1 the vaccine was produced from tumor cell cultures infected with Newcastle Disease Virus\textsuperscript{28} (14.2%).

**Treatment protocols**

The reviewed studies used different inclusion and exclusion criteria, treatment protocols and tumor infiltration identification techniques. Walker included patients with surgically accessible malignant glioma (WHO grade III or IV), with ECOG (Eastern Cooperative Oncology Group) performance status of 0, 1 or 2 and absence of other significant disease or pregnancy. There was no specific treatment protocol followed prior to vaccination and patients could receive the vaccine with or without chemotherapy or radiotherapy following surgery\textsuperscript{27}. Yamanaka, included patients with HGGs and Karnofsky scores varying from 30 to 80 who received vaccination following radiological confirmation of disease progression\textsuperscript{23}. Steiner, studied patients with Karnofsky scores greater than 60 all of whom had undergone maximum tumor resection and radiotherapy before receiving the vaccine\textsuperscript{28}. A similar protocol was used in the study of Liau which included GBM patients receiving surgical resection and external beam radiotherapy prior to vaccination\textsuperscript{22}. In the study of Prins, the “Stupp” protocol was applied, comprising of surgical resection and concomitant temozolimide chemo radiation, prior to the vaccination\textsuperscript{26}. Lastly, Chang, recruited patients with newly diagnosed or recurrent HGGs, newly diagnosed patients received surgical resection with radiation therapy whereas patients with recurrent gliomas were treated solely with surgical resection\textsuperscript{24}.

**Immunohistochemical analysis**

Similar immunohistochemical techniques were used in all studies to identify tumor microenvironment infiltrating lymphocytes. Serial paraffin sections were cut and stained with antihuman antibodies against CD3, CD8, CD4, CD45, CD45RO and transforming growth factor-b2 in the study of Liau\textsuperscript{22}; versus p53, glial fibrillary acidic protein (GFAP), nestin,
CD3, CD4 and CD8 in the study of Chang\textsuperscript{24}; versus CD8, CD45RO and mouse anti-human in the study of Walker\textsuperscript{27}; versus CD3, CD4, CD8, CD20 and CD56 in the study of Yamanaka\textsuperscript{23}; versus CD3 and CD8 for the study of Prins\textsuperscript{26}; versus CD8, EGFR (epidermal growth factor) type III deletion, neurofilament protein, GFAP, platelet endothelial cell adhesive molecule in the study of Steiner\textsuperscript{28}; and CD8, CD45RO, CD20 and CD56 for the study of Yu\textsuperscript{25}. (Table 2).

**Lymphocytes infiltrating tumor microenvironment**

The presence of tumor infiltrating lymphocytes (TILs) and the significance of it was analyzed to various degrees in the included studies. Tumor samples were collected following radiologically proven progression/recurrence and analyzed with immunohistochemistry as mentioned above. Three studies (42.8\%) reported presence of TILs in the post–vaccination specimens that were not present in the pre–vaccination samples examined\textsuperscript{25,29,30}, whereas the remaining 4 (57.2\%) reported increase (to various extents) in the numbers of pre-existing lymphocytes following vaccination.

To summarize the specifics, Yu, reported the presence of cytotoxic CD8 lymphocytes as well as the memory CD45RO cells that were not present prior to vaccination in 3 out of 6 patients (50\%) who underwent re-operation for tumor progression\textsuperscript{25}. Liau reported a robust T-cell infiltration in 4 out of 8 patients (50\%) who underwent re-operation, mainly consisting of CD8 and CD45RO and to a lesser degree CD4 helper cells\textsuperscript{29}. Similar results were published by Yamanaka with a significant accumulation of CD8 and CD4 tumor infiltrating cells in 2 out of 7 (28.6\%) patients following vaccination, while no such increase was observed in five non-vaccinated patients who underwent re-operation\textsuperscript{30}. An increase in the number of already pre-existing infiltrating CD8 and CD45RO cells, in all 4 patients undergoing re-operation, was detected by Walker\textsuperscript{27}. Steiner also reported an up to six-fold increase in CD8 cytotoxic cells in vaccinated patients compared to very low numbers in the non-vaccinated group\textsuperscript{28}.

Interestingly, Chang, observed two major changes in tumor samples received pre- and post-vaccination. Firstly, a shift from perivascular to relatively diffuse TIL infiltration following vaccination and in addition a reversal in the CD4 and CD8 ratio with an increase in the number of CD8 TILs\textsuperscript{24}. Lastly, Prins, described increased infiltration with CD3 and CD8 lymphocytes following dendritic cell vaccination in patients that had undergone re-operation for progression\textsuperscript{26}.
Clinical outcome and correlation with tumor microenvironment infiltration

Most of the studies included here were originally designed to assess the feasibility and safety of the treatment. Most authors agreed that there was a benefit in terms of OS and progression free survival following peripheral DC vaccination with Liau et al. going into further detail describing their findings.

They reported that out of the 8 patients who underwent re-operation for progression, 4 survived for more than 30 months whilst 3 survived for less than a year and the remaining one had intermediate survival. The patients that survived more than 30 months had shown a robust CD3 and CD8 lymphocytic tumor infiltration that was not present in the pre-operative tissue samples. This infiltration was not observed in the tumor samples of the 3 patients who survived less than a year.29

Despite not directly correlating their outcomes with immune infiltration, all six remaining studies reported improved overall survival in the vaccination treatment group. Overall survival varied from 348 to 1077 days for the vaccination group. In the studies of Yu, Liau, Steiner and Yamanaka, comparison with control groups was performed which showed statistically significant differences between the two groups. Most interestingly, Prins et al. showed statistically significant improvement in overall survival, not only when comparing the vaccination group with the controls but also when comparing patients who received treatment at first diagnosis to those receiving treatment following recurrence (p=0.03)26. (Table 3).

The most relevant prognostic factors described in these studies, in keeping with the existing literature on high grade gliomas, were patients’ age, performance score and treatment protocols used. No information, however, was provided on other potentially relevant parameters such as tumor size, location or its molecular and genetic makeup.

Discussion

Immunotherapy for HGGs is a relatively new concept. Unlike HGGs, in other common types of cancer, such as ovarian, colorectal and melanoma, the presence of TILs is consistently related to better clinical outcome31-36. The relevant literature on DC-immunotherapy for HGGs is still limited and therefore the number of studies included in our review is small, potentially limiting any didactic conclusions. Nonetheless, data provide indication of the types of lymphocytes that infiltrate malignant gliomas following
vaccination, suggest that infiltration can be boosted and also imply a positive correlation between induced infiltration and survival.

There appears to be unanimous agreement on the type of tumor infiltrating cells that appear or increase in numbers following peripheral DC vaccination. These mainly include CD8 cytotoxic, CD45RO memory and to a lesser degree CD4 helper cells. The presence of CD8 and the absence of CD20 lymphocytes in peripherally vaccinated patients suggest a Th1 immune response initiation rather than Th2, without excluding preferential CD8 immigration though.

Most studies suggested improved clinical outcomes following immunotherapy, potentially indicating that the observed recruitment of immune cells in the tumor’s microenvironment could be the determining factor. Such increased survival rates due to increased lymphocytic infiltration would be in agreement with the study of Yang et al. who investigated the correlation between the presence of CD8 cells in the initial tumor specimen and overall survival. They found that 65 out of 108 patients (60.1%) who had extended survival (>403 days) had an intermediate to extensive T-cell infiltration (p<0.006). Several other studies, including the study of Brooks et al. showed positive correlation between lymphocytic infiltration and better clinical outcomes. In contrast though, a study by Safdari et al. who assessed the prognostic implications of lymphocytic infiltration in 342 patients with WHO grade III and IV gliomas, showed negative correlation between the presence of TILs and survival. Furthermore, the teams of Schiffer and Rossi reported no correlation between infiltration and survival in their studies. Such observation is hard to explain but glioblastoma molecular heterogeneity, varying treatment and sampling protocols, as well as the lack of lymphocytic sub-type classification (CD4, CD8, T-reg) across studies could have potentially led to these contradicting results.

In our view, the small number of studies together with the relative lack of detailed description of prognostic factors limits any firm conclusions on the relevance of lymphocytic tumor infiltration to survival. The quality of the data and particularly its consistency across a range of studies reviewed here, does however raise the possibility that DC immunotherapy may improve patient outcomes, warranting further studies. It is important to highlight at this point the heterogeneity of the patients included in the mentioned studies. With the exception of two, the remaining included both patients with newly diagnosed and recurrent malignant gliomas with overall survival data referring to the combination of the groups. Subgroup
analysis comparing recurrent to newly diagnosed disease was performed by two studies with contradicting results (Table 3). Additionally, comparison with control groups is not the result of randomized trials but a comparison to matched or historical controls.

Furthermore, data regarding the safety of the technique is limited. Authors report that the technique was well tolerated with the most commonly reported adverse events being headaches, fever, myalgia, fatigue, lymphopenia and seizures, in a small number of patients and no author has reported any grade 3 or 4 National Cancer Institute Common Toxicity Criteria adverse events. The above indicate that conclusions regarding survival rates, safety and recommendation or not of the technique cannot be safely drawn and that the results of ongoing phase III trials should be awaited.

Although it is well established that glioblastoma induces immunosuppression, the exact mechanisms and patient specific factors underlying those processes remain unclear. Great emphasis is now given on the presence of T regulatory cells that have been shown to downregulate CD4 and CD8 cells and also produce IL-10 and TGB-b which block effector T cell response44,45. Treatment related immunosuppression investigated by Authier et al indicated that treated glioma cells were more immunosuppressive and formed tumors at a faster rate in vitro and in an animal models, compared to untreated ones44. Interestingly, increased apoptotic rates of lymphocytes were first described by Walker et al. who demonstrated that T cells expressing Fas ligand (Fas-L, CD95L) were eight times more susceptible to apoptosis compared to those not expressing Fas ligand37.

Liau proposed that active tumor recurrence and/or bulky residual tumor further negatively influence the ability of T lymphocytes to accumulate within the local tumor microenvironment affecting infiltration following DC vaccination22. Notably, Yu implied that T cell infiltration is a characteristic of a subset of patients that undergo re-operation25,45 whereas Prins found a positive correlation between increased T lymphocytic infiltration and the mesenchymal gene expression26. Controversially, a recent study by Yang et al. showed that dendritic cells loaded with autologous tumor lysate increased tumor angiogenesis and indicated for the first time that the latter process could potentially promote tumor progression46. Of note though, the study was based on an animal model and there is no evidence to date indicating that similar findings would be expected in human cancer and more specifically glioblastoma. Nonetheless, the findings create an important question for future research.
The most important finding of this review is the wide variation in the degree of response to DC-immunotherapy measured by the increase in the numbers of infiltrating lymphocytes. The variations could arguably be attributed to the different protocols followed by each study group but there were significant differences even within individual studies with some patients exhibiting significant, some moderate and some no infiltration at all. The above observations could indicate that there are intrinsic factors, specific to each patient, or group of patients, that determine their response to treatment. Genetic, immunological and molecular profiling of patients, ideally from peripheral blood, tumor tissue or CSF could potentially help identify the characteristics of those patients who are more likely to respond to the treatment as well as help develop patient specific treatment protocols.

In the face of these challenges, dendritic cell immunotherapy for high grade gliomas, has attracted significant interest from the scientific community. There are currently two ongoing phase III trials, namely DCVax which uses activated monocytes loaded with antigens from the patient’s own tumor tissue, and ICT-107 which uses dendritic cells, pulsed with six synthetic peptides derived from tumor associated antigens from glioblastoma cells. At least five more phase one and two trials, due for completion between 2017 and 2020 are also currently running (from http://clinicaltrials.gov).

Future studies are likely to aim to maximize success of immunotherapy by specifically catering for the peculiarities of the central nervous system and the immunosuppressive properties of glioblastoma. Thus various techniques that can amplify dendritic cell based, tumor specific, immune response, are currently under investigation. Targeted patient selection (pSTAT signaling, mesenchyman gene expression signature, TCR)\textsuperscript{47,48}, administration of adjuvant treatments (tetanus, polyICLC, imiquimod)\textsuperscript{26,49}, as well as novel tumor antigen generation and delivery strategies (CMV RNA, cocktail of peptides)\textsuperscript{50,51} are being trialed aiming to improve the specificity and the efficiency of the technique.

Conclusion

Within the limitations imposed by small numbers and potential biases, we reviewed the literature in an attempt to evaluate the degree of immune cell infiltration of malignant glioma microenvironment induced by peripheral DC immunotherapy. Present data indicate that tumor infiltration by lymphocytes is feasible, that it can be induced/boosted by DC immunotherapy and that this may positively affect clinical outcome. It still remains unclear
which factors enhance or restrict the phenomenon and therefore determine patients’ response to treatment. However, current evidence create numerous opportunities for further research, not only to characterize the types of immune changes following immunotherapy but also to identify the patients that will benefit from it the most.

**Abbreviations and acronyms**
- GBM (glioblastoma multiforme)
- CNS (central nervous system)
- MHC (major histocompatibility complex)
- OS (overall survival)
- DC (dendritic cell)
- HGGs (high grade gliomas)
- CD (cluster of differentiation)
- ID (intradermally)
- IT (intrathecally)
- SC (subcutaneously)
- TILs (tumor infiltrating lymphocytes)
- EGFR (epidermal growth factor)
- GFAP (glial fibrillary acidic protein).

**Disclosure of potential conflict of interests**
No potential conflicts of interest were disclosed.
Reference list


Table 1. Main Characteristics of Included Studies

<table>
<thead>
<tr>
<th>Authors</th>
<th>Country</th>
<th>Trial phase</th>
<th>Number of patients</th>
<th>Mean Age</th>
<th>Vaccine preparation</th>
<th>Vaccination Route</th>
<th>Administration of adjuvants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yu et al. 2004</td>
<td>U.S</td>
<td>I</td>
<td>14</td>
<td>44.7</td>
<td>DC + TL</td>
<td>I.D</td>
<td>No</td>
</tr>
<tr>
<td>Chang et al. 2011</td>
<td>Taiwan</td>
<td>I/II</td>
<td>17</td>
<td>44.7</td>
<td>DC + AITC</td>
<td>S.C</td>
<td>No</td>
</tr>
<tr>
<td>Liau et al. 2005</td>
<td>U.S</td>
<td>I</td>
<td>12</td>
<td>48.9</td>
<td>DC + AETP</td>
<td>I.D</td>
<td>No</td>
</tr>
<tr>
<td>Prins et al. 2010</td>
<td>U.S</td>
<td>I</td>
<td>23</td>
<td>51</td>
<td>DC + TL</td>
<td>I.D</td>
<td>Yes - imiquimod or poly-ICLC</td>
</tr>
<tr>
<td>Walker et al. 2007</td>
<td>Australia</td>
<td>I</td>
<td>13</td>
<td>51</td>
<td>DC + AITC</td>
<td>I.D</td>
<td>No</td>
</tr>
<tr>
<td>Steiner et al. 2004</td>
<td>Germany</td>
<td>I/II</td>
<td>23</td>
<td>49.8</td>
<td>VMATC</td>
<td>I.D</td>
<td>Yes - NDV</td>
</tr>
<tr>
<td>Yamanaka et al. 2005</td>
<td>Japan</td>
<td>I/II</td>
<td>24</td>
<td>48.9</td>
<td>DC + TL</td>
<td>I.D and/or I.T</td>
<td>No</td>
</tr>
</tbody>
</table>

I.D: intradermally, S.C: subcutaneously, I.T: intratumourally, D.C: dendritic cell, TL: Tumour Lysate, TP: Tumour Peptides, AITC: autologous irradiated tumour cells, AETP: acid-eluted tumour peptides, VMAT: virus modified autologous tumour cells, NDV: Newcastle Disease Virus
Table 2. Markers used for immunochemistry, reported treatments and performance score prior to initial vaccination, use of corticosteroids during vaccination treatment and histological diagnosis.

<table>
<thead>
<tr>
<th>Study</th>
<th>Immunohistochemistry markers</th>
<th>Pre-vaccination treatment</th>
<th>Performance score</th>
<th>Corticosteroids</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chang</td>
<td>CD3, CD4, CD8, GFAP, Nestin</td>
<td>Rx(60Gy) for new diagnosis only</td>
<td>KPS: 70-90 (mean: 83)</td>
<td>Yes, up to 20mg/day</td>
<td>GBM(x14), MO(x2), AA(x1)</td>
</tr>
<tr>
<td>Liau</td>
<td>CD3, CD4, CD8, CD45, CD45R0, TGF-B2</td>
<td>Rx(60GY) only(x9), Tamoxifen(x2), SRS(x1)</td>
<td>KPS: 60-100 (mean: 87)</td>
<td>Off for two weeks prior to vaccination</td>
<td>GBM(x12)</td>
</tr>
<tr>
<td>Prins</td>
<td>CD3, CD8</td>
<td>TMZ(x17), TMZ+ lomustine(x2), Irinotecan+bevacizumab(x1), erlotinib+TMZ(x1), TMZ+Gliadel(x1), TMZ+NVD(x1)</td>
<td>KPS: 60-100 (mean: 84)</td>
<td>No or 10 days off prior to vaccination</td>
<td>GBM(x23)</td>
</tr>
<tr>
<td>Walker</td>
<td>CD4, CD45R0</td>
<td>Nil(x5), RecHG(x5), prev LGG(x2), Rx+TMZ (x1)</td>
<td>ECOG: 0-2 (no further information)</td>
<td>Not reported</td>
<td>GBM(x9), AA(x4)</td>
</tr>
<tr>
<td>Steiner</td>
<td>CD8, GFAP, EGFR type III del.</td>
<td>Rx(60Gy)</td>
<td>KPS: 70-100 (mean: 70.4)</td>
<td>Off steroids at time of vaccination – no window reported</td>
<td>GBM(x23)</td>
</tr>
<tr>
<td>Yamanaka</td>
<td>CD3, CD4, CD8, CD20, CD56</td>
<td>Rx(60Gy), nitrosurea based chemotherapy</td>
<td>KPS: 30-100 (mean: 62.5)</td>
<td>Not reported</td>
<td>GBM(x18), AA(x6)</td>
</tr>
<tr>
<td>Yu</td>
<td>CD8, CD20, CD45RO, CD56</td>
<td>Nil(x7), Gliadel(x1), hydroxyurea(x1)</td>
<td>KPS: 60-100 (mean: 93)</td>
<td>Off steroids at time of vaccination – no window reported</td>
<td>GBM(x7), AA(x2)</td>
</tr>
</tbody>
</table>
Table 3. Survival rates and subgroup analysis

<table>
<thead>
<tr>
<th>Study</th>
<th>OS Vaccination Group</th>
<th>OS Control Group</th>
<th>Type of Control</th>
<th>Statistical significance</th>
<th>OS Newly diagnosed GBM</th>
<th>OS Recurrent GBM</th>
<th>Ongoing Disease at time of vaccination</th>
<th>Stable disease at time of vaccination</th>
<th>Proneural Gene Expression</th>
<th>Mesenchymal Gene Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yu</td>
<td>31.0 months</td>
<td>7.0 months</td>
<td>Matched controls</td>
<td>0.001</td>
<td>No subgroup analysis</td>
<td>No subgroup analysis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Chang</td>
<td>17.3 months</td>
<td>12.6 months</td>
<td>Historical controls</td>
<td>-</td>
<td>12.7 months&lt;sup&gt;1&lt;/sup&gt;</td>
<td>32.2 months&lt;sup&gt;1&lt;/sup&gt;</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Liau</td>
<td>23.4 months</td>
<td>18.3 months</td>
<td>historical controls</td>
<td>P=0.006</td>
<td>-</td>
<td>11.7 months</td>
<td>35.8 months</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Prins</td>
<td>31.4 months</td>
<td>-</td>
<td>-</td>
<td></td>
<td>35.9 months</td>
<td>17.9 months</td>
<td>-</td>
<td>-</td>
<td>P=0.664&lt;sup&gt;2&lt;/sup&gt;</td>
<td>P=0.0046&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Walker</td>
<td>11.6 months</td>
<td>-</td>
<td>-</td>
<td></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steiner</td>
<td>23.3 months</td>
<td>11.4 months</td>
<td>non selected controls</td>
<td>P&lt;0.001</td>
<td>-</td>
<td>No recurrent GBM included</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yamanaka</td>
<td>16.0 months</td>
<td>13.3 months</td>
<td>non selected controls</td>
<td>P=0.010</td>
<td>No newly diagnosed patients included</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

OS: overall survival
All studies included both patients with newly diagnosed and recurrent GBM with the exceptions of Steiner and Yamanaka. OS data refer to both groups combined.

1. Figures refer to mean 5-year survival in the subgroup analysis.
2. Subgroup analysis showing that vaccinated patients with proneural gene expression did not have significantly better survival compared to 60 control proneural gene expression tumors whereas vaccinated patients with mesenchymal gene expression had significantly extended survival compared to 82 control mesenchymal gene expression tumors.
3. Chang and Prins performed subgroup analysis dividing vaccinated patients to newly diagnosed and recurrent GBM.
4. Liau performed subgroup analysis dividing vaccinated patients to those with ongoing disease at time of vaccination and stable disease regardless whether they were newly diagnosed or recurrent. P=0.006 refers to the combined group (newly diagnosed and recurrent) compared to controls.
Figure 1. Schematic illustration showing the production, administration and in vivo activation of DC vaccines for the treatment of high grade gliomas.