Autologous T cell Therapy for Cytomegalovirus as a Consolidative Treatment for Recurrent Glioblastoma

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Running Title: CMV-specific immunotherapy for glioblastoma

Keywords: adoptive immunotherapy, recurrent GBM, cytomegalovirus, phase I clinical trial, safety
Financial support: National Health and Medical Research Council of Australia (NH&MR; Project ID: APP1020325); Rio-Tinto Ride to Conquer Cancer Flagship Program. RK is supported by a Senior Principal Research Fellowship from NH&MR.

Conflicts of interest: Authors declare no conflict of interest.

Weight count: Abstract: 231; Text: 3177

Number of figures and tables: 7
Abstract

Glioblastoma multiforme (GBM) is one of the most aggressive human brain malignancies. Even with optimal treatment, median survival is less than six months for patients with recurrent GBM. Immune-based therapies have the potential to improve patient outcome by supplementing standard treatment. Expression of human cytomegalovirus (CMV) antigens in GBM tissues provides the unique opportunity to target viral antigens for GBM therapy. Here we report findings of a formal clinical assessment of safety and potential clinical efficacy of autologous CMV-specific T cell therapy as a consolidative treatment for recurrent GBM. From a total of 19 recurrent GBM patients, CMV-specific T cells were successfully expanded from 13 patients (68.4%) and 11 of whom received up to four T cell infusions. Combination therapy based on T cell infusion and chemotherapy was well tolerated and we detected only minor adverse events. The overall survival of these patients since first recurrence ranged from 133 to 2428 days with a median overall survival of 403 days. Most importantly, four out of 10 patients that completed the treatment remained progression free during the study period. Furthermore, molecular profiling of CMV-specific T cell therapy from these patients revealed distinct gene expression signatures which correlated with their clinical response. Our study suggests that that a combination therapy with autologous CMV specific T cells and chemotherapy is a safe novel treatment option and may offer clinical benefit for recurrent GBM patients.
Introduction

Glioblastoma multiforme (GBM) is one of the most malignant human adult brain tumours. Current treatment regimes include surgical resection, radio- and chemotherapy, but patient prognosis remains poor with a median survival after initial diagnosis of less than 15 months (1) and a 5-year survival rate of less than 10% (2). GBM is incurable and inevitably recurs after initial therapy. Median survival for recurrent GBM is 3-6 months and most patients do not survive longer than one year (2-4). While chemotherapy, especially the introduction of temozolomide, has been shown to increase survival to some degree(1), dramatic improvements in outcome for patients with GBM have remained elusive. Therefore, much interest has focused on immunotherapeutic approaches. Strategies under investigation include tumour lysate vaccines, tumour antigen vaccines and targeting of immunomodulatory molecules (reviewed in (5)).

Accumulating evidence indicates human cytomegalovirus (CMV) as a contributing factor to glioma progression (6, 7) and CMV has been suggested as a therapeutic target(8). While not classified as an oncogenic virus, CMV can increase cellular proliferation, angiogenesis and immune evasion, thus enabling several hallmarks of cancer (9, 10). Recently, an onco-accessory function of CMV has also been described in a mouse model of glioblastoma using murine cytomegalovirus infection (11). More importantly, CMV antigens and nucleic acids have been detected in histological sections of GBM but not in surrounding healthy tissue(12-18). Although these histological findings have been disputed (19-21), more recent studies have confirmed the presence of CMV sequences in malignant cells (22, 23). Vaccination of
one patient with dendritic cells pulsed with autologous GBM lysate elicited a CMV-specific immune response, further supporting the presence of CMV antigens in GBM tissue (24). This provides an opportunity to target viral antigens with immune-based therapies. Low levels of CMV antigen expression in tumour cells were found to be associated with longer survival of GBM patients (15, 16), thus indicating that antiviral therapy could improve GBM prognosis. Additionally, recent studies supplementing standard GBM therapy with antiviral valganciclovir treatment for more than 6 months demonstrated a survival benefit for GBM patients (25, 26).

We have explored the feasibility and safety of an autologous T-cell based GBM immunotherapy targeting CMV antigens. We have recently shown that CMV-specific CD8+ T-cells in GBM patients have reduced functional capacity, but that this limitation can be reversed following in vitro stimulation. Adoptive transfer of these cells into a single patient with recurrent GBM in combination with standard chemotherapy was associated with long term disease-free survival (27). Here, we report the findings from a formal clinical assessment of this initial finding as a Phase I clinical trial. We demonstrate that autologous CMV-specific T-cell therapy is safe with minimal side effects and may offer clinical benefit for recurrent GBM patients.
Materials and Methods

Study design, Ethics and patients

This phase I clinical study was designed to assess the safety and tolerability of autologous CMV-specific T-cell therapy for recurrent GBM. This clinical trial was conducted according to Declaration of Helsinki principles and was approved by The QIMR Berghofer Medical Research and Uniting Care Health Human Research Ethics Committees. All participants signed a consent form which was approved by both ethics committees. This study is registered under the Australia New Zealand Clinical Trial Registry (ACTRN12609000338268). Study completion required a minimum of three T cell infusions, whereas additional infusions could be administered depending on availability of cells. Infusions consisting of 25 - 40 x 10⁶ autologous CMV-specific T-cells in sterile saline were administered in 4 (+/- 2) week intervals. The infusions were coordinated with periods of chemotherapy to avoid unwanted side effects. Peripheral blood samples collected prior to each infusion and at regular intervals post-infusion were used for haematological and immunological monitoring. Each follow-up visit included vital observations and a quality-of-life questionnaire. Magnetic resonance imaging (MRI) was used to assess baseline tumour load prior to infusion and at regular intervals after first infusion.

In vitro expansion of CMV-specific T-cells from GBM patients

CMV-specific T-cells were generated by in vitro stimulation with synthetic peptide epitopes. Peripheral blood mononuclear cells (PBMCs) were isolated from peripheral blood by Ficoll gradient, washed and resuspended in RPMI-1640 supplemented with 10% FBS (growth medium). The cells were co-cultured with autologous PBMCs pre-
sensitized with CMV peptides (5μg/ml; Suppl Table S1) at a responder to stimulator ratio of 2:1 in growth medium. After 3 days and then every 3 to 4 days thereafter, the cultures were supplemented with growth medium containing 120IU/ml recombinant interleukin-2 (IL-2; Komtur Pharmaceuticals). Cells were cryopreserved on day 14, after testing for sterility and CMV-specific T-cells using an IFN-γ intracellular cytokine assay. Release criteria were ≥ 0.1% CMV specific T-cells in total lymphocyte population and >50% cell viability.

**Characterization of CMV CTL by intracellular cytokine assay and flow cytometry**

PBMCs or cultured T-cells were stimulated with peptides corresponding to defined CD8⁺ T-cell epitopes derived from CMV proteins (1μg/ml) and incubated in the presence of BrefeldinA for 4 hours. For polyfunctional analysis, cells were incubated with a CD107a-antibody, BrefeldinA and Monensin for 5h. After surface staining for CD8, CD4 and CD3, cells were fixed and permeabilized with cytofix/cytoperm and stained for IFNγ (and IL-2 and TNF in polyfunctional assays). Immune monitoring was achieved by surface staining with appropriate pMHC multimers (Immudex) and surface markers, followed by fixation with a transcription buffer set and intracellular staining (all reagents from BD Biosciences, unless otherwise indicated). Stained cells were resuspended in PBS containing 2% paraformaldehyde and acquired using a FACSCanto II or LSR Fortessa with FACSDiva software (BD Biosciences). Post-acquisition analysis was conducted using FlowJo software (TreeStar).

**Cell sorting and gene expression analysis by Taqman RT PCR array**
PBMCs were revived from cryopreserved stocks and stained with appropriate CMV-dextramers, followed by staining for surface markers to allow for separation of monocytes, B cells and T-cells. After filtering the cell suspension through a nylon mesh for removal of cell clumps, total CD8+ T-cell and CMV specific CD8+ T-cell populations were isolated using a FACS Aria (BD Biosciences) cell sorter. Total RNA was purified using Qiagen RNeasy Micro kit. An RNA amount equivalent to 3000 cells was transcribed into cDNA using the high-capacity-RNA-to-cDNA-Kit. The cDNA was preamplified with a custom primer pool, loaded into custom designed taqman array cards and run on the Viia7 real-time PCR system (all reagents from Life Technologies). The array cards were designed to contain 93 genes that were described to be regulated in CD8 T-cells during CMV infection(28). Data was normalized to housekeeping genes (18S, actin, β2-microglobulin) and analyzed using Gene Spring v12.5 software.

**Statistical analysis**

Mortality and progression-free survival were characterised using Kaplan-Meier curves, and the corresponding median survival times were obtained for the intention-to-treat (ITT) and per-protocol (PP) patient populations of 11 and 10 patients, respectively. The ITT patient population included patients who received at least one T-cell infusion and the PP patient population included patients who received at least three infusions. Time to death or censoring was defined as the time between the last follow-up date and the date of the first recurrence. Time to progression or censoring was defined as the time between the date of the subsequent recurrence after the treatment or the last follow-up date and the date of the first recurrence. Left truncations occurred since patients
did not receive the treatment at the date of the first recurrence and have been accounted for appropriately. The analyses were performed using SAS Enterprise Guide 4.3 and R 3.0.1.
Results

Patient Characteristics

Nineteen patients with recurrent GBM were recruited for this study. Eligibility criteria for the study included (a) Age 18 years or above; (b) Geographically accessible for follow up; (c) ability to provide informed consent; (d) ECOG performance status 0, 1, 2 or 3; (e) life expectancy of at least 3 months (f) positive CMV serology and (g) Previous histological diagnosis of GBM (WHO grade IV) and radiological and/or clinical evidence of tumour progression or recurrence. Four patients had to be withdrawn before venesection due to progressive disease, while insufficient CMV-specific T cells were expanded from two patients due to low precursor frequency or poor cell viability. All patients received standard treatment with maximal safe surgical debulking at primary diagnosis, external beam radiotherapy and chemotherapy (Table 1). CMV-specific T-cells were successfully expanded from 13 patients, but two patients had to be withdrawn due to progressive illness and one patient discontinued the intervention after two infusions due to progressive disease. In total, 10 patients completed a minimum of three infusions as required per protocol (Table 2).

In vitro expansion and functional characterization of CMV-specific CD8+ T-cells

Autologous CMV-specific T-cells were successfully expanded from 13 out of 19 patients (Table 2). Ex vivo analysis of CMV-specific T-cells from GBM patients using peptide-MHC (pMHC) multimers showed antigen-specific T-cell frequencies ranging from 0.026% to 18.4% of total CD8+ T-cells (median 4.2%). Following in vitro stimulation with HLA-matched CMV peptide epitopes, a significant increase in antigen-specific T-cells was observed (range 4.2% to 92.7% of total CD8+ T-cells; median
49.85%; p<0.0001, Fig. 1A and B and Suppl Table S2). Phenotypic characterization showed that these T cells were predominantly CD3\(^+\) with a combination of both CD8\(^+\) and CD4\(^+\) T-cells (Fig. 1C). The majority of CMV-specific T-cells were CD45RA\(^-\)CD27\(^+\) and/or CD57\(^+\) a phenotype characteristic of effector cells (Fig. 1D). Intracellular cytokine analysis revealed that high proportions of these in vitro expanded CD8\(^+\) T-cells expressed IFN\(_\gamma\) in response to stimulation with CMV epitopes (Fig. 1E and F). Furthermore, in-depth functional analysis of these T cells from some patients showed that these cells displayed polyfunctional profile and also expressed CD107 (data not shown).

**Safety and clinical evaluation of CMV-specific T-cell-based immunotherapy for recurrent GBM**

Of the 13 patients for whom CMV-specific T cells were generated, 10 patients received three to four T cell infusions (2-4 x 10\(^7\) cells/infusion, Table 2). One patient only received two infusions, whereas two patients died prior to the availability of T cells. Infusions were generally well tolerated and mostly minor adverse events were recorded (Table 3). The toxicity grading was assigned according to the National Cancer Institute Common Terminology Criteria for Adverse Events. A single serious adverse event (SAE) possibly related to T-cell therapy was recorded (Patient GBM:19). This patient had a generalized seizure within 12 hours of the first T-cell infusion and was hospitalized for three days. The patient had a history of seizures prior to entering into the current trial. The investigators discussed this SAE with the Data Safety Monitoring Committee, and deemed the SAE was unlikely to be associated with T-cell therapy. No further seizures developed in this patient after subsequent T-cell infusions. These
analyses indicate that autologous CMV-specific T-cell infusions are a safe treatment for GBM. The median overall survival (OS) of the 11 patients that received at least one infusion was 403 days (range: 133 to 2428 days, Fig. 2A). The time to progression for all patients after infusion ranged from 108 to more than 1783 days, with a median of 246 days (Fig. 2B). Of the 11 patients treated with T cell therapy, four patients remained progression-free (Table 2). Patient GBM:01 showed the longest stabilization of disease with almost four years of progression-free survival (PFS) after the T-cell infusion. This patient had no other treatment subsequent to CMV-specific T-cell therapy and remains disease-free to date.

**Immunological and molecular analysis of CMV-specific T-cell therapy**

To assess the efficiency of T-cell therapy, we first determined the effect of antigen-specific T-cell frequencies on survival. However, there was no correlation between the number of CMV-specific T cells transferred and the overall survival and progression-free survival (Fig 3A & B). *Ex vivo* longitudinal analysis of CMV-specific T cells showed that although in some patients there was a small increase in the precursor frequency of CMV-specific T-cells, the number of antigen-specific T cells returned to baseline after the completion of adoptive immunotherapy (suppl. Fig. S1A & B). The functional profile of these T-cells also remained unchanged during and after the completion of T-cell therapy (suppl. Fig. S1C).

We next explored the possibility that gene expression profiling of T-cell therapy may help to distinguish patients that might benefit from adoptive immunotherapy. To test this hypothesis, we used a custom array which allowed quantitative expression analysis of different categories of genes relevant for T cell function (Suppl. Table S3).
This expression profiling revealed that 47 out of 93 genes were significantly changed in CMV-specific CD8\(^+\) T-cells following *in vitro* expansion (Fig. 3C and suppl. Fig. S2A and B). This gene expression profile was consistent with a signature of activated T-cells and was further confirmed by specific antibody staining and flow cytometry (suppl. Fig. S2C).

To identify gene expression patterns that might be of prognostic value, we divided our patient cohort into two groups: (a) GBM patients who had short PFS (<100 days) and (b) GBM patients who either remained progression-free within the study period or developed progressive disease after more than 100 days. In depth analysis of gene expression data revealed that these two groups of patients showed significant differences in the expression of seven genes including T-cell transcription factors (*Eomes, BCL6* and *FoxP3*), cytokine/chemokines (*IFNG* and *CCL5*) and checkpoint markers (*CTLA4* and *XAF1*); (Fig. 3D & E). These analyses suggest that expression profiling of T-cell therapy may provide clues on the potential therapeutic benefit of adoptive immunotherapy.

*CMV specific T-cells are present in GBM tumour tissue and show a distinct phenotype compared to peripheral blood*

*Ex vivo* analysis of tumour infiltrating antigen-specific T-cells can provide some critical insights on the immune control of malignant cells. In our study, GBM:16 patient who received four infusions of CMV-specific T-cells developed progressive disease four months after the completion of T-cell infusions and then underwent surgical tumour resection. We isolated T-cells from the resected tumour tissue and were able to detect CMV-specific CD8\(^+\) T-cells (Fig. 4A, upper panel). However, the majority of these
antigen-specific T-cells failed to express multiple cytokines including IFN-γ, TNF, IL-2 and showed poor cytotoxic activity as assessed by CD107a mobilization following stimulation with CMV peptides (Fig. 4A, lower panel). Furthermore, staining of CD103 as a marker for tissue resident T-cells (T_{rm}) revealed that while approximately one third of CD8^+ T-cells in the tumour tissue were T_{rm}, none of the CMV-specific T-cells expressed CD103 (Fig. 4B). The frequency of CMV-specific T-cells in the tumour tissue was approximately four-fold lower when compared to T-cells circulating in peripheral blood at different time points before (d0), during (d35) and after T-cell therapy (d78 and d121, Fig. 4C). Tumour infiltrating CMV-specific T-cells expressed higher levels of PD-1, TIM-3 and CTLA-4 and lower levels of transcription factors T-bet, Eomes and LEF-1 (Fig. 4C). These observations suggest that tumour infiltrating antigen-specific T-cells in this GBM patient displayed poor functional capacity and increased expression of inhibitory receptors when compared to T-cells from peripheral blood. Similar expression patterns of PD-1, CTLA-4, TIM-3 and transcription factors were detected in the global CD8^+ T cell population (suppl. Fig. 3). We further detected almost 5-fold higher levels of regulatory T-cells (CD4^+CD25^+FoxP3^+) than in peripheral blood which is consistent with an immunosuppressive environment in tumour tissue (Fig. 4C).
Discussion

The survival of patients with recurrent GBM remains poor despite use of all currently available cytotoxic therapeutics (1, 29-32). Over the last decade immune-based therapies have emerged as possible tools for the treatment of recurrent GBM (33-35) and exploratory studies have shown improved PFS and OS (36-38). In 2002, Cobbs and colleagues demonstrated expression of the CMV proteins IE-1 and late antigen in GBM tumour biopsies which were later confirmed by other groups (10, 12-14, 21). Further studies have suggested that CMV-encoded proteins such as viral IL-10 and US28, a G-protein-coupled receptor-like protein, may act as tumour promoters in GBM (10, 39, 40). The presence of CMV in GBM has generated considerable interest, especially the potential targeting of the viral proteins using immune-based therapies (18). We have shown previously that CMV-specific T-cells from the majority of GBM patients display reduced multifunctional potentiality and that in vitro stimulation of these T-cells can improve their functional profile (27). Adoptive transfer of these T-cells into one recurrent GBM patient was shown to be safe with possible clinical benefit.

In the present study we report the outcome of the first clinical trial for adoptive immunotherapy using CMV-specific T-cells in recurrent GBM patients. We recruited 19 recurrent GBM patients and of these 11 patients received multiple infusions of autologous in vitro expanded CMV-specific T-cells. A number of important conclusions can be drawn from this study. First, adoptive transfer of CMV-specific T-cells was completely safe with minimal toxicities. Although the CMV-specific T-cell therapy was provided in combination with standard therapies (Table 2), we did not observe any deleterious impact of these therapies on the adoptively transferred CMV-specific T-
cells. More importantly, patients did not experience any severe side effects and the only recorded SAE was deemed unrelated to the treatment.

Second, clinical follow up analyses showed that CMV-specific immunotherapy was coincident with disease stabilization and prolonged progression-free survival in some patients. The median OS in our study was >57 weeks (range: 19-346 weeks) with a median PFS of >35 weeks (range: 15.4-254 weeks). Most importantly, four of the 10 patients who completed T-cell therapy remained progression-free. Although promising, these observations will require confirmation in a formal Phase II randomised clinical trial. Interestingly, the positive effects of antiviral therapy in the GBM setting have also recently emerged from a randomized, placebo-controlled study investigating the use of the antiviral drug valganciclovir for the treatment of primary gliomas (25).

Third, we were unable to see any correlation of antigen-specific T-cell frequencies following adoptive immunotherapy and clinical outcome. Although some patients showed a small increase in virus-specific T-cells in the peripheral blood following the first few infusions, this effect was transient. Furthermore, phenotypic and functional analysis showed no link between the clinical response and antigen-specific T-cells in the peripheral blood. Although the reason for this lack of correlation is unknown, it is probable that the tumour microenvironment and disease burden may impact on T-cell function and influence the clinical response to adoptive immunotherapy (41, 42). Indeed, preliminary data from a single patient (GBM:16) who developed progressive disease soon after the completion of adoptive immunotherapy showed that although antigen-specific T-cells were detected in the tumour, the majority of these cells lacked multifunctional potentiality and had undetectable
expression of CD103 which is a crucial marker for tissue residence. In addition, these T-cells showed increased expression of checkpoint inhibitory receptors when compared to the circulating effector cells from peripheral blood, potentially reflecting the impact of the tumour microenvironment. We further detected higher amounts of regulatory T cells which might contribute to immunosuppression in GBM tissues (43). These observations were supported by gene expression analysis of antigen-specific T-cells used for adoptive immunotherapy. Indeed increased prolonged PFS was co-incident with lower expression of checkpoint inhibitory receptors and increased expression of T-cell transcription factors crucial for T-cell function. It is important to emphasize that these observations will require more in-depth analyses in a larger cohort of patients within a randomized Phase II clinical study.

Taken together, adoptive immunotherapy of recurrent GBM patients with CMV-specific T-cells is safe and may provide long-term clinical benefits. These studies provide an important platform for a formal assessment of adoptive T-cell immunotherapy in both therapeutic and prophylactic settings. CMV-specific T-cell-based immunotherapy should be considered as a consolidative treatment following primary diagnosis of GBM for the prevention of recurrent disease.
Acknowledgements

This study was supported by the National Health and Medical Research Council of Australia (NH&MRC) Australia (Project ID: APP1020325) and the Rio-Tinto Ride to Conquer Cancer Flagship Program. RK is supported by a Senior Principal Research Fellowship from NH&MRC.

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Data analysis and interpretation: Andrea Schuessler, Corey Smith, Glen Boyle, Kerenaftali Klein, Rajiv Khanna

Manuscript writing and revision: All Authors

Final approval of manuscript: All authors
References


Figure Legends

Figure 1: Characterisation of *in vitro* expanded CMV-specific CD8+ T-cells and clinical response to adoptive T cell therapy. (A) Representative FACS plot of pMHC-multimer staining of CMV-specific CD8+ T-cells before and after *in vitro* expansion with CMV-derived synthetic peptide epitopes (Suppl Table S1). (B) Pair-wise analysis of *in vitro* expanded CMV-specific CD8+ T-cells before and after stimulation with CMV-derived synthetic peptide epitopes (data from 12 of 13 GBM patients is shown; no suitable HLA-peptide multimer was available for one patient). (C & D) Phenotypic analysis of *in vitro* expanded T-cells. These cells were incubated with antibodies specific for various surface markers (CD14, CD19, CD56, CD3, CD4, CD8, CD27, CD57 and CD45RA) and then analysed using LSR Fortessa with FACSDiva software. (E & F) Analysis of IFN-γ expression by *in vitro* expanded CMV-specific CD8+ T-cells measured by ICS assay. Panel E shows representative data from one recurrent GBM patient. Panel F shows pair wise analysis of IFNγ expression by CMV-specific CD8+ T-cells before and after *in vitro* stimulation. These T-cells were incubated with the relevant peptide epitopes (Suppl Table S2) in the presence of BrefeldinA for 4 hours. Cells were then incubated with antibodies specific for CD3, CD4 and CD8, and then assessed for intracellular IFN-γ production.

Figure 2: (A) Overall survival of GBM patients treated with autologous CMV-specific T cells since first recurrence. (B) Progression-free survival
following CMV-specific T-cell adoptive immunotherapy (determined from the date of the first T-cell infusion).

**Figure 3:** Molecular analysis of *in vitro* expanded T cells and correlation to clinical outcome. (A & B) Correlation of the CMV-specific T-cell infusion dose with overall survival and time to progression. Data represents the correlation between the numbers of adoptively transferred CMV-specific T cells received by each patient, as determined using the intracellular IFN-γ analysis, and the overall survival since first recurrence and time to progression following the first T-cell infusion. (C) Gene expression profiling of CMV-specific T-cells before and after *in vitro* expansion. Heat map of 47 out of 93 significantly different genes is shown. This analysis is based on T-cells from ten GBM patients enrolled in the study. Each column represents one patient. Data was normalized to housekeeping genes (18S, actin, β2-microglobulin) and analyzed using Gene Spring v12.5 software. (D) Heat map of selected genes which were differentially expressed in T-cells used for adoptive immunotherapy in patients with short (<100 days) or long-term (>100 days) progression-free survival. Blue line above heatmap indicates patients <100 days, red line indicates patients >100 days. (E) Fold change expression analysis of selected genes in T-cell therapy from short or long-term progression-free surviving patients.
Figure 4: *Ex vivo* analysis of tumour infiltrating and peripheral blood circulating CMV-specific CD8$^+$ T-cells from a GBM patient. (A) *Ex vivo* HLA-peptide multimer staining and polyfunctional analysis of tumour infiltrating CMV-specific T-cells from patient GBM:16. (B) Expression of CD103 (a marker of tissue-resident T-cells) on total CD8$^+$ and CMV-specific T-cells in tumour infiltrating lymphocytes. (C) Longitudinal comparative phenotypic analysis of peripheral blood circulating and tumour infiltrating CMV-specific CD8$^+$ T-cells and CD4$^+$ Tregs. Tumour infiltrating lymphoid cells and PBMC were incubated with HLA-peptide multimers, antibodies specific for CD3, CD4, CD8 and specific markers (as indicated on the Y-axis of each box) and then analysed using using a LSR Fortessa with FACSDiva software. Post-acquisition analysis was conducted using FlowJo software.
Table 1: Characteristics of GBM patients and treatment history prior to T-cell therapy

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<tr>
<td>15</td>
<td>60</td>
<td>m</td>
<td>GBM</td>
<td>197</td>
<td>1</td>
<td>283</td>
<td>1</td>
<td>0</td>
<td>yes</td>
<td>None</td>
</tr>
<tr>
<td>16</td>
<td>50</td>
<td>m</td>
<td>GBM</td>
<td>989</td>
<td>1</td>
<td>1092</td>
<td>1</td>
<td>0</td>
<td>yes</td>
<td>None</td>
</tr>
<tr>
<td>17</td>
<td>23</td>
<td>m</td>
<td>GBM</td>
<td>436</td>
<td>1</td>
<td>-</td>
<td>1</td>
<td>0</td>
<td>yes</td>
<td>Avastin</td>
</tr>
<tr>
<td>18</td>
<td>61</td>
<td>f</td>
<td>GBM</td>
<td>323</td>
<td>NIA</td>
<td>-</td>
<td>2</td>
<td>0</td>
<td>yes</td>
<td>None</td>
</tr>
<tr>
<td>19</td>
<td>41</td>
<td>f</td>
<td>GBM</td>
<td>413</td>
<td>2</td>
<td>704</td>
<td>3</td>
<td>0</td>
<td>yes</td>
<td>Carboplatin, Lomustine, Avastin</td>
</tr>
</tbody>
</table>

Time to recurrence is calculated from date of first surgery to date of tumour recurrence based on MRI findings. Number of operations refers to tumour debulking surgeries only. Time to T-cell therapy is calculated from date of first diagnosis. Asterisks indicate patients that were initially diagnosed with low-grade malignancies. Shaded areas indicate patients that did not complete the trial. Abbreviations: d, days; AA, anaplastic astrocytoma; XRT, Radiotherapy; TMZ, Temozolomide chemotherapy; NIA, No information available.
Table 2: Clinical follow up of adoptive T-cell therapy of recurrent GBM patients

<table>
<thead>
<tr>
<th>GBM:ID</th>
<th>Total cells expanded</th>
<th>Number of cells per infusion</th>
<th>Number of infusions</th>
<th>Time to progressive disease after first infusion (d)</th>
<th>Treatment in addition to T-cells</th>
<th>Follow up since first infusion (d)</th>
<th>Current status (31.12.2013)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>26.8x10^7</td>
<td>3x10^7</td>
<td>4</td>
<td>no progression</td>
<td>TMZ</td>
<td>1447</td>
<td>alive</td>
</tr>
<tr>
<td>02</td>
<td>11.8x10^7</td>
<td>2.8x10^7</td>
<td>4</td>
<td>57</td>
<td>Avastin</td>
<td>330</td>
<td>deceased</td>
</tr>
<tr>
<td>03</td>
<td>7.4x10^7</td>
<td>2.0x10^7</td>
<td>3</td>
<td>135</td>
<td>Avastin, Thalidomide</td>
<td>1134</td>
<td>alive</td>
</tr>
<tr>
<td>04</td>
<td>14.6x10^7</td>
<td>2.9x10^7</td>
<td>4</td>
<td>no progression</td>
<td>TMZ</td>
<td>1010</td>
<td>deceased*</td>
</tr>
<tr>
<td>05</td>
<td>failed</td>
<td>N/A</td>
<td>0</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>deceased</td>
</tr>
<tr>
<td>06</td>
<td>22x10^7</td>
<td>4x10^7</td>
<td>4</td>
<td>36</td>
<td>Avastin, TMZ</td>
<td>153</td>
<td>deceased</td>
</tr>
<tr>
<td>07</td>
<td>16.8x10^7</td>
<td>3.5x10^7</td>
<td>2</td>
<td>withdrawn prior to 3rd infusion</td>
<td>Avastin, Carboplatin</td>
<td>35</td>
<td>deceased</td>
</tr>
<tr>
<td>09</td>
<td>13x10^7</td>
<td>2.5x10^7</td>
<td>4</td>
<td>no progression</td>
<td>TMZ</td>
<td>462</td>
<td>alive</td>
</tr>
<tr>
<td>11</td>
<td>13.7x10^7</td>
<td>N/A</td>
<td>0</td>
<td>withdrawn before infusion</td>
<td>N/A</td>
<td>N/A</td>
<td>deceased</td>
</tr>
<tr>
<td>12</td>
<td>failed</td>
<td>N/A</td>
<td>0</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>deceased</td>
</tr>
<tr>
<td>13</td>
<td>15.1x10^7</td>
<td>3.2x10^7</td>
<td>4</td>
<td>34</td>
<td>Avastin, CCNU</td>
<td>100</td>
<td>deceased</td>
</tr>
<tr>
<td>14</td>
<td>10.6x10^7</td>
<td>N/A</td>
<td>0</td>
<td>withdrawn before infusion</td>
<td>N/A</td>
<td>N/A</td>
<td>deceased</td>
</tr>
<tr>
<td>15</td>
<td>9.2x10^7</td>
<td>2.5x10^7</td>
<td>3</td>
<td>287</td>
<td>none</td>
<td>317</td>
<td>deceased</td>
</tr>
<tr>
<td>16</td>
<td>19.4x10^7</td>
<td>4x10^7</td>
<td>4</td>
<td>143</td>
<td>TMZ, surgery</td>
<td>392</td>
<td>alive</td>
</tr>
<tr>
<td>19</td>
<td>13.7x10^7</td>
<td>3x10^7</td>
<td>3</td>
<td>no progression</td>
<td>Avastin, Lomustine</td>
<td>175</td>
<td>alive</td>
</tr>
</tbody>
</table>

Shaded areas indicate patients that underwent venesection but did not complete the trial. Abbreviations: d, days; N/A, not applicable; TMZ, Temozolomide chemotherapy; CCNU, Lomustine chemotherapy; *patient death was not related to GBM
Table 3: Safety assessment following adoptive T-cell therapy of recurrent GBM patients*

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>Number of Patients Affected (Attribution Score**)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Grade 1: Mild</strong></td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Visual Hallucination</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Pyrexia of Unknown Origin</td>
<td>1 (2)</td>
</tr>
<tr>
<td>High Blood Pressure</td>
<td>1 (2)</td>
</tr>
<tr>
<td><strong>Grade 2: Moderate</strong></td>
<td></td>
</tr>
<tr>
<td>Abnormal Liver Function Tests</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Seizure</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Anxiety</td>
<td>1 (2)</td>
</tr>
<tr>
<td><strong>Grade 3: Severe</strong></td>
<td></td>
</tr>
<tr>
<td>Seizure</td>
<td>1 (2)</td>
</tr>
</tbody>
</table>

*Severity grade and attribution scores assessed according to the NCI Common Terminology Criteria for Adverse Events. **Attribution score: 1 = unrelated, 2 = unlikely, 3 = possible, 4 = probable, 5 = definite. Adverse Event Logs were compiled from clinical observation (including vital signs), patient interview and blood samples taken during infusions and at follow up visits. Events scored as unrelated were excluded from this table.
Fig. 1

A. Pre expansion vs. post expansion for pMHC-multimer binding in CD8+ cells.

B. Graph showing percent CD8+ pMHC-multimer+ cells with a P<0.0001.

C. Scatter plot of % positive for various cell markers.

D. Scatter plot of % positive for CD45RA+ and CD45RA-CD27+CD95+ cells.

E. No peptide vs. + peptide for IFNγ production in CD8+ cells.

F. Graph showing % CD8+ IFNγ+ cells before and after peptide stimulation.
A

Range: 133-2498 days
Median OS: 403 days

B

Range: 108-1853 days
Median PFS: 246 days
Pre expansion

Post expansion

Fig. 3
A

GBM:16

2.75%

CD8

pMHC-multimer

B

Total CD8

HCMV specific CD8

unstained

stained

CD103

2.25%

2.01%

32.9%

2.72%

CD8

C

% CD8+ pMHC-multimer+

MFI PD-1

% TIM-3

MFI CTLA-4

MFI T-bet

MFI Eomes

MFI IFN-γ

% Treg

- peripheral blood Dec 12 (d0)
- peripheral blood Jan 13 (d35)
- peripheral blood Feb 13 (d78)
- peripheral blood Apr 13 (d121)
- brain May 13

Fig. 4
Autologous T cell Therapy for Cytomegalovirus as a Consolidative Treatment for Recurrent Glioblastoma

Andrea Schuessler, Corey Smith, Leone Beagley, et al.

Cancer Res  Published OnlineFirst May 4, 2014.

Updated version  Access the most recent version of this article at: doi:10.1158/0008-5472.CAN-14-0296
Supplementary Material  Access the most recent supplemental material at: http://cancerres.aacrjournals.org/content/suppl/2014/05/01/0008-5472.CAN-14-0296.DC1
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