OncoImmunology 3, e29381; June 2014; © 2014 Landes Bioscience

Cellular immunotherapy directed against human cytomegalovirus as a novel approach for glioblastoma treatment

Andrea Schuessler[1], David G Walker[2], and Rajiv Khanna[1,*]

[1]QIMR Centre for Immunotherapy and Vaccine Development and Tumour Immunology Laboratory; Department of Immunology; QIMR Berghofer Medical Research Institute; Brisbane, QLD Australia; [2]Newro Foundation; The Wesley Hospital; Brisbane, QLD Australia

Keywords: cytotoxic T cells, adoptive immunotherapy, recurrent glioblastoma multiforme, cytomegalovirus, clinical trial

Glioblastoma multiforme (GBM) has a very poor prognosis, despite multimodal therapy including surgery, radiation and chemotherapy. A novel adoptive immunotherapy that exploits the presence of cytomegalovirus antigens in malignant brain cancer cells has been shown to be safe and elicit potential clinical benefit for the treatment of recurrent GBM.

Cytomegalovirus Antigens as New Targets for Immunotherapy of GBM

Glioblastoma multiforme (GBM) is a very aggressive form of human brain cancer with a low median survival of only slightly more than one year.[1] Despite surgery, chemotherapy and radiation, GBM inevitably recurs and life expectancy is reduced to approximately six months. There is an urgent need for new treatment options that can improve patient outlook. Immunotherapies, both cellular and antibody based approaches, have great potential and are the focus of intense research.[2] The discovery of cytomegalovirus (CMV) antigens in GBM tissues but not surrounding healthy brain cells,[3] provided an exciting opportunity to boost pre-existing antiviral immunity to combat this brain malignancy. While CMV is not considered an oncogenic virus, it can promote several hallmarks of cancer, including deregulation of the cell cycle, immune evasion and stimulation of angiogenesis.[4] The precise role of CMV in the context of GBM is still debated and remains an area of ongoing study. A recent study has shown direct killing of primary GBM cells by autologous CMV-specific T cells,[5] which provides further rationale for the use of antiviral T cells for GBM therapy. Our work has focused on developing a novel adoptive immunotherapy approach targeting CMV antigens for patients with recurrent GBM.

Adoptive Immunotherapy is Safe with Potential Clinical Benefit

We have shown previously that CMV specific cytotoxic T cells derived from seropositive GBM patients can be expanded in the laboratory following in vitro stimulation with synthetic CMV epitopes. Adoptive transfer of these cells into 1 patient with recurrent GBM was shown to be safe and coincident with long-term survival.[6] We have now completed a formal assessment of CMV-specific immunotherapy as a Phase I clinical trial and provided autologous T-cell therapy to 10 patients with recurrent GBM[7] (**Fig. 1**). This treatment was administered in 3 to 4 doses and proved to be completely safe with only mild to moderate side effects like headaches or fatigue. The overall survival since initial diagnosis ranged from 133 d to 2498 d with a median of 403 d. Most importantly, 4 out of the 10 patients that received at least 3 T-cell infusions, remained cancer free during the study period.[7] The patient with the longest disease stabilization has not shown signs of tumor relapse to date, which is more than 4 y after receiving T-cell therapy. For the patients that did have tumor recurrence after T-cell therapy, we reported a median progression-free survival of 246 d, which is considerably longer than the expected life expectancy of 6 mo. While conclusions about survival need to be confirmed in a larger, randomized trial, the results are promising and indicate CMV may be a useful target for GBM therapy.

Molecular Profiling of T-cell Therapy Might Provide Prognostic Signatures

Predicting response to treatment or identification of markers that influence treatment efficacy could be highly beneficial for the clinical management of patients. To identify such prognostic signatures, we performed a comprehensive immunological and molecular profiling of antigen-specific T cells isolated from GBM patients.[7] Gene expression analysis of the T-cell product used for infusion detected major changes compared with ex vivo T cells that were consistent with a signature of activation. Further

*Correspondence to: Rajiv Khanna; Email: rajiv.khanna@qimr.edu.au
Submitted: 05/27/2014; Accepted: 05/27/2014; Published Online: 06/25/2014
Citation: Schuessler A, Walker DG, Khanna R. Cellular immunotherapy directed against human cytomegalovirus as a novel approach for glioblastoma treatment. OncoImmunology 2014; 3:e29381; http://dx.doi.org/10.4161/onci.29381

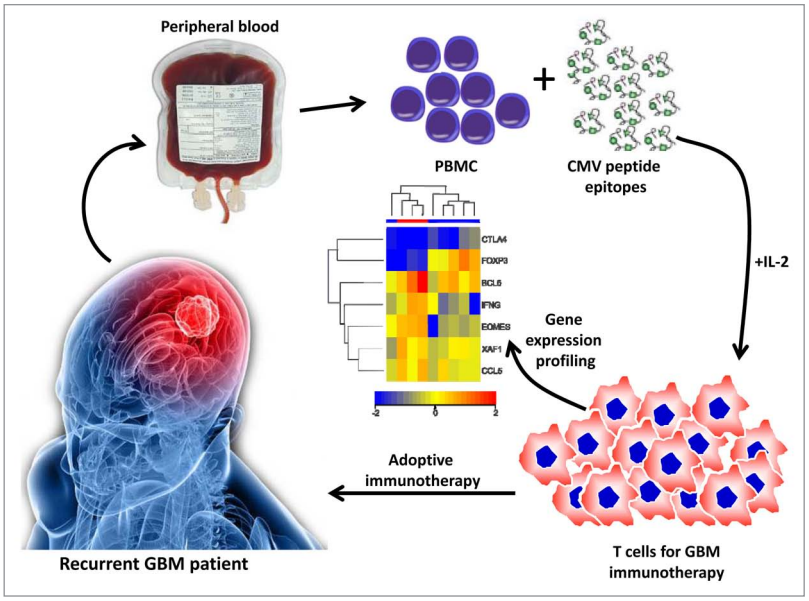

Figure 1. Generation of antigen-specific T cells for adoptive therapy of GBM patients using MHC class I and/or class II-restricted peptide epitopes from cytomegalovirus antigens. Peripheral blood mononuclear cells (PBMC) are purified from 200–400 mL of patient peripheral blood, stimulated with cytomegalovirus (CMV) peptide epitopes and incubated for 14 d in the presence of interleukin-2 (IL-2). Following microbiological testing, the CMV-specific T cells are ready to be reinfused into the patient within 4 wk after the blood is drawn. In addition, these in vitro expanded T cells are also used for gene expression profiling to determine potential correlates of clinical response.

in-depth analysis revealed a signature of 7 genes that distinguished long-term disease free survivors from patients that relapsed more rapidly (**Fig. 1**). These genes were associated with T-cell effector function, potentially indicating that a more functionally active T-cell product is more effective in controlling cancer reoccurrence. Albeit preliminary, these results indicate prognostic biomarkers might be found by molecular analysis of T cells used for immunotherapy. Immunological and molecular analysis of peripheral blood T cells before and after immunotherapy failed to reveal any major changes that could have been indicative of response to treatment. Interestingly, tumor-infiltrating lymphocytes (TILs) isolated from a patient who relapsed after completing 4 T-cell infusions, displayed a distinct phenotype compared with T cells derived from peripheral blood of the same donor. This phenotype was consistent with local immunosuppression in the tumor which might contribute to treatment failure. This finding suggests that immunological profiling of lymphocytes in the tumor microenvironment could be an important tool to predict response to treatment and patient prognosis.

In summary, we have completed the first clinical assessment of CMV-specific adoptive immunotherapy for recurrent GBM. Autologous T-cell therapy was completely safe and coincided with extended progression free survival in 4 out of 10 patients. This study provides an important platform for further evaluation of cellular immunotherapy in the GBM setting, potentially in combination with other therapeutics.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, Belanger K, Brandes AA, Marosi C, Bogdahn U, et al.; European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups; National Cancer Institute of Canada Clinical Trials Group. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 2005; 352:987-96; PMID:15758009; http://dx.doi.org/10.1056/NEJMoa043330
2. Reardon DA, Wucherpfennig KW, Freeman G, Wu CJ, Chiocca EA, Wen PY, Curry WT Jr., Mitchell DA, Fecci PE, Sampson JH, et al. An update on vaccine therapy and other immunotherapeutic approaches for glioblastoma. Expert Rev Vaccines 2013; 12:597-615; PMID:23750791; http://dx.doi.org/10.1586/erv.13.41
3. Cobbs CS, Harkins L, Samanta M, Gillespie GY, Bharara S, King PH, Nabors LB, Cobbs CG, Britt WJ. Human cytomegalovirus infection and expression in human malignant glioma. Cancer Res 2002; 62:3347-50; PMID:12067971
4. Soroceanu L, Cobbs CS. Is HCMV a tumor promoter? Virus Res 2011; 157:193-203; PMID:21036194; http://dx.doi.org/10.1016/j.virusres.2010.10.026
5. Nair SK, De Leon G, Boczkowski D, Schmittling R, Xie W, Staats J, Liu R, Johnson LA, Weinhold K, Archer GE, et al. Recognition and Killing of Autologous, Primary Glioblastoma Tumor Cells by Human Cytomegalovirus pp65-Specific Cytotoxic T Cells. Clin Cancer Res 2014; 20:2684-94; PMID:24658154; http://dx.doi.org/10.1158/1078-0432.CCR-13-3268
6. Crough T, Beagley L, Smith C, Jones L, Walker DG, Khanna R. Ex vivo functional analysis, expansion and adoptive transfer of cytomegalovirus-specific T-cells in patients with glioblastoma multiforme. Immunol Cell Biol 2012; 90:872-80; PMID:22508289; http://dx.doi.org/10.1038/icb.2012.19
7. Schuessler A, Smith C, Beagley L, Boyle GM, Rehan S, Matthews K, Jones L, Crough T, Dasari V, Klein K, et al. Autologous T-cell Therapy for Cytomegalovirus as a Consolidative Treatment for Recurrent Glioblastoma. Cancer Res 2014;In Press; PMID:24795429; http://dx.doi.org/10.1158/0008-5472.CAN-14-0296