

Temozolomide as a vaccine adjuvant in GBM

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ABSTRACT

RESULTS

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Background: Cytotoxic chemotherapy that induces lymphopenia is predicted to ablate the benefits of active antitumor immunization. Temozolomide (TMZ) is an effective chemotherapeutic for patients with glioblastoma multiforme (GBM), but it induces significant lymphopenia.

Methods: In a Phase II trial, patients with newly-diagnosed, completely resected GBM are vaccinated with an EGFRvIII-specific peptide (CDX-110) q2 weeks x 3 after radiation (XRT) (~60Gy) and TMZ (75mg/m²/d) and then monthly with 5 day TMZ cycles (200mg/m²/d).

Results: TMZ induces transient Grade 3 lymphopenia (< 500 cells/μL) in 70% of patients after the first TMZ cycle with nadirs occurring 14-21 days after treatment (n=10). Regulatory T-cell (T_{Reg}) (CD4+CD25+CD45RO+FOXP3+) levels increased from 5.2±1.5% (3.3 – 7.6) to 11.8±2.6% (6.9 – 13.8) (P=0.0004; paired t-test) with TMZ and XRT and averaged 12.2±4.0% (6.4 – 18.1) after the second TMZ cycle (P=0.007) (n=6). Despite these findings, in patients assessed, both humoral and cellular EGFRvIII-specific immune responses appear to be enhanced with TMZ. In the first clinical trial, ACTIVATE patients were vaccinated with an EGFRvIII-specific peptide q2 weeks x 3 after radiation (XRT) and TMZ (75mg/m²/d) median TTP was 64.5 weeks and median survival was 126.1 weeks. In a second trial, ACT II, patients received vaccinations as above following (XRT) and TMZ (75mg/m²/d) and then monthly with 5 day TMZ cycles (200mg/m²/d). Median survival and TTP after vaccination is 22.1 weeks with no patients progressing (n=8).

Conclusions: Despite conventional dogma, we demonstrated that both chemotherapy and immunotherapy can be delivered concurrently without negating the effects of immunotherapy. TMZ-induced lymphopenia may prove to be synergistic with a peptide vaccine.

INTRODUCTION

Despite aggressive surgical resection, high-dose focused radiation therapy, and chemotherapy, patients diagnosed with GBM have a median survival time of 14 months after diagnosis. Several clinical trials with selected glioma patients, involving vaccinating them with dendritic cells and either acid-eluted peptides or an antigen-specific peptide, have demonstrated promising results. Temozolomide (TMZ), a methylating chemotherapeutic, has recently been shown to prolong survival in patients with GBM and has become part of the standard regimen used to treat them, but TMZ also often induces a profound and long-lasting lymphopenia that may limit such promising and specific immunotherapeutic approaches.

Multiple preclinical model systems have demonstrated that the depletion of immune cell subsets can abrogate the efficacy of several types of immunotherapeutic approaches, suggesting that chemotherapy administered during the effector stages of immunotherapy may be deleterious. However, this does not preclude using these agents together when appropriately timed to minimize the aforementioned effects. Furthermore, the depletion of certain suppressive lymphocyte subsets, such as regulatory T-cells (T_{Reg}), may be a highly desirable outcome of chemotherapy, including TMZ, yielding greater immunotherapeutic efficacy or possibly promoting a desirable cytokine profile for adequate tumor control.

Clinical Study Schema

Two trials targeting the EGFRvIII tumor specific antigen are in progress at Duke University and M.D. Anderson Cancer Center. These trials enroll patients with newly-diagnosed GBM that are EGFRvIII positive.

ACTIVATE trial: patients received radiation and concurrent TMZ followed by vaccination with EGFRvIII-specific peptide/GM-CSF.

ACT II trial: patients received the same concurrent radiation and TMZ, but received the vaccination on day 21 of repetitive 28-day TMZ cycles.

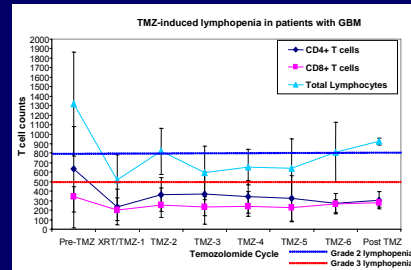
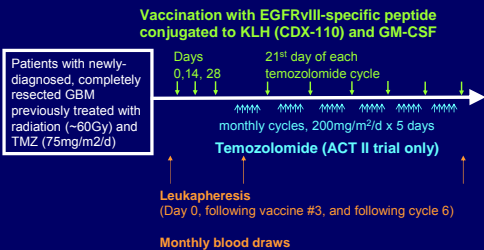


Fig. 1. Peripheral blood counts were monitored in patients with newly-diagnosed GBM (n=10) prior to initiation of standard treatment and then monthly during administration of TMZ (50-75 mg/kg per day) for six weeks during external beam radiotherapy followed by 28 day cycles of TMZ (150-200 mg/kg per day for five days). CD4⁺ T-cells, CD8⁺ T-cells, and total lymphocytes were measured by the Duke University Clinical Hematology Laboratory and absolute counts plotted over time. Grade 2 lymphopenia (blue dashed line above; Common Toxicity Criteria™, <800 lymphocytes/μl of blood) was induced in all patients receiving TMZ and Grade 3 lymphopenia (red dashed line above; CTC; <500 lymphocytes/μl of blood) was induced in 7/10 patients after the first cycle of TMZ. Lymphocyte counts returned to normal levels after cessation of TMZ treatment. These results demonstrate that standard dose TMZ induces transient but profound lymphodepletion in the majority of patients with GBM.

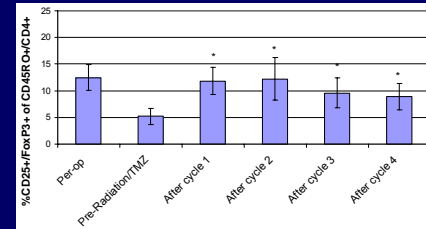


Fig. 2. TMZ enhances T_{Reg} recovery. T_{Reg} (%CD25+foxp3+ of CD45RO+CD4⁺ T cells) levels pre-operatively, post-operatively prior to TMZ and immediately prior to each TMZ cycle. After staining PBMC surface antigens (CD45RO-FITC, CD25-PE, and CD4-PerCP-Cy5.6, BD #555432, #555432, and #341654 respectively) cells are washed extensively and incubated on ice for 30 minutes in fixation/permeabilization buffer (eBioscience, #5123-43). Cells are then washed in 1X permeabilization buffer (eBioscience, #8333-56), pelleted, and stained with foxp3-APC (e-Bioscience, #17-4776-73, clone PCH101). CD25⁺foxp3⁺ are gated from CD45RO⁺CD4⁺ lymphocytes. Values are an average of six donor sets.

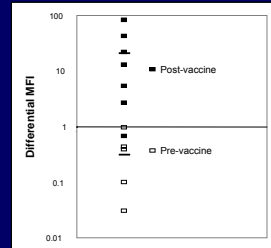


Fig. 3. Maximal humoral response of pre and post vaccine serums indicated by level of human antibody binding to EGFRvIII immobilized beads. Differential MFI indicates difference between serum sample and serum sample pre-adsorbed with EGFRvIII-specific peptide. EGFRvIII was immobilized on magnetic particles (Dyna Bead M280, #142.03 tosylactivated beads). Human anti-peptide antibody is captured during incubation of serum or plasma with the beads. Captured antibody was detected through the binding of a labeled secondary anti-human polyclonal antibody. Particles were then analyzed on a flow cytometer. To ensure specificity, separate serum samples were pre-adsorbed with EGFRvIII peptide to block specific antibody from binding to particles. Anti-peptide antibody levels are determined from the differential between blocked and unblocked samples.

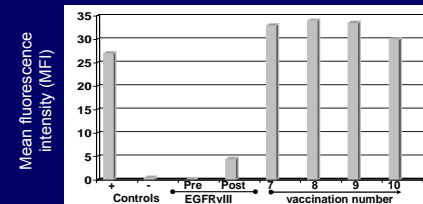


Fig. 4. Flow cytometric analysis of humoral responses induced in the peripheral blood of a patient with GBM vaccinated with a peptide spanning the mutated version of the epidermal growth factor receptor, EGFRvIII. Before the patient received any vaccine (pre), there was no detectable humoral response to EGFRvIII, but after the third vaccination, there was a marked induction of EGFRvIII IgG-specific responses (post). These induced humoral responses did not appear to be diminished during the sequential administration of TMZ.

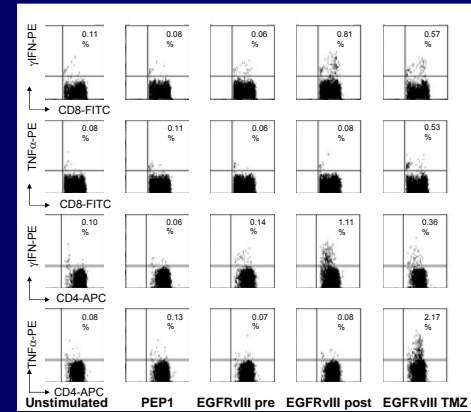


Fig. 5. Flow cytometric analysis of EGFRvIII-specific CD4⁺ and CD8⁺ T-cell responses induced in the peripheral blood of a patient with GBM. To further clarify whether the temozolomide would impact the induced CD8⁺ cytotoxic responses to EGFRvIII, the patient's PBMCs from each leukapheresis and monthly pre-TMZ blood collections were stimulated with an irrelevant peptide, PEP-1 (HDTYYCVGKNKELE) (10μg/mL) as a negative control, or EGFRvIII peptide (10μg/ml). The induced immune responses were specific to the components of the vaccine and were sustained despite the sequential administration of temozolomide. As anticipated gamma-IFN responses were initially detected but later the patient developed EGFRvIII-specific TNF-α responses as well.

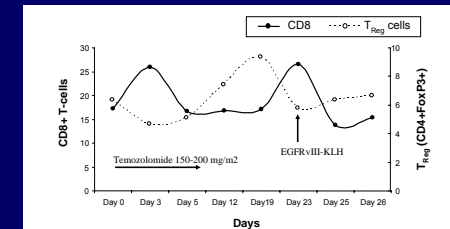


Fig. 6. Flow cytometric determination of the absolute percentage of CD8⁺ T-cells (gated on total lymphocytes) and T_{Reg} (CD4⁺CD25⁺FoxP3⁺) cells (gated on CD4⁺ T-cells) in the peripheral blood of a GBM patient during the course of treatment with temozolomide and immunotherapy. The vaccine was administered on day 23 during this particular cycle.

Time to Progression

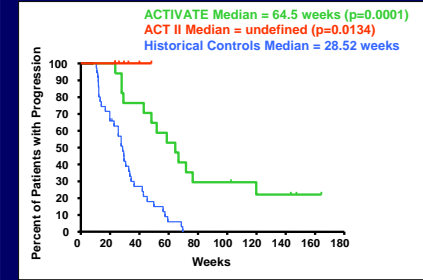


Fig. 7. The Historical control group is matched for degree of resection, age, functional status, and EGFRvIII status.

Survival

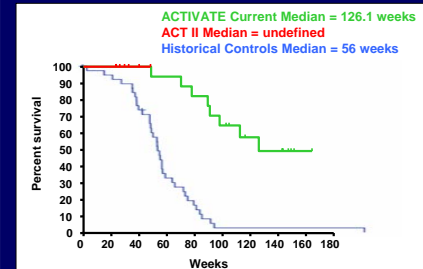


Fig. 8. Kaplan-Meier survival curves. The ACTIVATE study has a median survival time of 126.1 weeks which compares favorably with the historical control (56 weeks) and the published results of Gliadel (59.6 weeks) Westphal et al, 2003, Temodar (58.4 weeks), Stupp et al, NEJM 2005 and EGFRvIII-positive patients (55 weeks), Pelloski et al, JCO June 1, 2007.

DISCUSSION

Concurrent antitumor active immunization is not contraindicated during chemotherapy with TMZ in patients having GBM. We present several findings that indicate that the co-administration of the TMZ has not affected the efficacy of a EGFRvIII-targeted vaccine. EGFRvIII-specific CD3⁺CD8⁺γ-IFN producing T-cells induced by vaccination, do not appear to be diminished during cycles of concurrently administered TMZ. In addition, EGFRvIII-specific IgG responses are induced and maintained while the concurrent TMZ. Finally, we have followed the CD8⁺ T-cell and T_{Reg} populations during a single treatment cycle and found that there appears to be a window of T-effector (CD8⁺ T-cell) responsiveness with a relative diminution of T_{Reg} although, overall, TMZ may increase T_{Reg} recovery. Thus the concurrent administration of TMZ during active immunization, in the manner we described, does not appear to diminish the induced immune responses.

In conclusion, this preliminary experience suggests that sequential administration of chemotherapy and immunotherapy may not be deleterious; however additional patients are needed for confirmation of our findings.