Mahaley Clinical Research Award: Chemosensitization of Glioma through Dendritic Cell Vaccination

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SUMMARY

A major reason chemotherapy fails in cancer treatment is drug resistance. New targets against chemotherapy resistance have been developed with the identification of molecular pathways in drug resistance. These targets are proteins that are highly expressed in human gliomas and are known to be tumor antigens. The immune system produces specialized white blood cells called dendritic cells (DCs). DCs are the most potent antigen-presenting cell of the immune system. DCs have demonstrated the ability to stimulate antibodies and cell-mediated immune responses against tumor antigens. Immunotherapy has emerged as a novel treatment strategy for gliomas with tumor antigens serving as the driving force. Clinical immunotherapy trials for glioma patients using vaccinations made of tumor antigens combined with dendritic cells ex vivo have shown promising results. DC vaccinations may increase sensitivity to chemotherapy, as demonstrated by a significant increase in 2-year survival rates in patients with malignant gliomas who received chemotherapy after immunotherapy (51). The use of DC vaccinations to increase sensitivity of tumor cells to chemotherapy can be rationalized as a novel strategy. Hence, this review will focus on the recent advances in the identification of tumor-associated antigens in gliomas, as well as their biological function related to drug resistance. The current research status and the future direction of DC vaccines to treat glioma in animal models and clinical trials will also be discussed.

INTRODUCTION

Increases in median survival in patients with glioblastoma multiforme remain modest despite recent advances in surgery, chemotherapy, and radiation therapy. To date, the best known treatment for the most aggressive and malignant brain tumor, glioblastoma multiforme, increases median survival by only 2 to 3 months. Chemotherapy resistance by malignant tumor cells is a major reason for this modest response to therapy. Resistance to chemotherapy is due to either an innate property of malignant tumor cells or by their ability to acquire resistance during drug treatment. Researchers over the past decade have been successfully decoding the mystery behind the mechanism of drug resistance in tumor cells and have begun to pave the road to understanding the molecular mechanisms by which brain tumor cells develop a drug-resistant phenotype.[9] Fas antigen (Fas) and Fas ligand has been shown to participate in cytotoxicity mediated by T lymphocytes and natural killer cells. Drug resistance in ovarian cancer was overcome as shown by Wakahara et al.[50] in 1997, by using the combination of anti-Fas Ab and various drugs. Efficient elimination of both intrinsically resistant myeloma cells and acquired multiple drug resistance (MDR) tumor cells was shown with granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-12 (IL-12) expressing tumor cell vaccines in animal models.[44] In addition, some drug resistant tumor cells expressed significantly higher HLA class I surface antigens and TAP messenger ribonucleic acid (mRNA) than drug-sensitive cells, which indicate that drug-resistant tumors are probably more readily lysed by MHC-restricted, tumor-associated CTLs.[16,33] Extensive investigations of intracellular vaccinations targeting molecules related to drug resistance have been performed.[40] Immunotherapy is demonstrating, through collected evidence, to be an effective approach in overcoming a major treatment barrier in cancer treatment-drug resistance with chemotherapy. Generating an effective anti-tumor immune response is limited in many cancer immunotherapy trials. However, newer DC-based approaches have been successful in generating an effective anti-tumor immune response. In fact, targeting of tumor-associated antigen TRP-2 by DC vaccination was demonstrated for the first time by Dr. Liu and his colleagues to significantly increase chemotherapeutic sensitivity. Immunotherapy not only induces T cell cytotoxicity, as is well established, but can also make tumors more sensitive to drug therapy.[29] The synergistic effect of immunotherapy along with cancer chemotherapy is gaining evidence and popularity as a promising adjunct to cancer therapy for brain tumors.[51]

DENDRITIC CELLS ARE THE MOST POTENT ANTIGEN-PRESENTING CELLS

Cytotoxic T Cells, as established by a strong body of evidence, plays a vital role in mounting an effective anti-

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tumor immune response.[12,19,22] The presence of tumor antigen is necessary to generate effective tumoricidal T cell immunity. T cell activation, clonal expansion, and exertion of cytolytic effector function follows the introduction of naïve T cells to tumor antigen. Patients with malignant gliomas have been shown to have a faulty ability to mount an effective anti-tumor immune response. This is due largely to tumor cells' amplified immunosuppressive chemokines which depresses native antigen presenting cells' ability to recognize, ingest, and process tumor derived antigens.[22,53,57] Effective cytotoxic T cell effector function is dependent on effective antigen presentation; thus, the establishment of a viable immunotherapeutic approach to the treatment of malignant gliomas requires a strategy that successfully introduces tumor antigens to T cells in vivo. DC-based vaccines are a promising treatment strategy that elicits tumor specific antigen presentation to the immune system. Many co-stimulatory molecules are abundantly expressed on DCs. These co-stimulatory molecules are essential for effective activation of naïve T cells and possess the ability to efficiently process and present antigenic peptides in combination with cell-surface MHC. The most potent of the APCs are the DCs. DCs are capable of initiating cytolytic T cell function in vitro and in vivo.[6] Due to recent advances in DC biology, we are now able to generate large numbers of DCs in vitro where normally, in circulation, DCs are present only in extremely small numbers.[46] DCs, derived in vitro from PBMCs, can be primed against tumor specific antigens in culture and, upon subsequent vaccination in tumor-bearing hosts, have the ability to elicit anti-tumor immunity in a variety of neoplastic models including lymphoma, melanoma, prostate and renal cell carcinoma.[20,25,35,47] The efficacy of a peripherally administered tumor-derived peptide pulsed DC vaccine in generating anti-tumor cytotoxic immunity was first demonstrated by Siesjo[45] in a rodent glioma model. A correlation between the development of antigen-specific T cell responses and a favorable clinical outcome was shown in the DC vaccine study in melanoma.[5]

DC VACCINATIONS INDUCE ANTIGEN-SPECIFIC CYTOTOXIC T CELLS IN GLIOMA PATIENTS

Liau et al.[27] first described the successful treatment of established intracranial gliomas in rats treated with tumor-peptide pulsed DC vaccination after successful reports describing the efficacy of DC-based vaccination in extracranial experimental neoplastic models.[32] In a Phase I clinical trial involving patients with newly diagnosed high grade glioma, Yu et al.[55] described the use of a DC vaccine in nine patients with newly diagnosed glioma (seven with GBM and two with AA). Three intradermal vaccinations of DC pulsed ex vivo with autologous tumor cell surface-derived peptides isolated by means of acid elution of cultured tumor cells were administered to the nine patients. Assessing vaccine-elicited generation of tumor-specific cytotoxic immunity was performed to determine therapeutic efficacy. PBMC isolated from patients before vaccination and at various time points after the initiation of therapy, were restimulated in vitro by re-exposure to autologous tumor targets and then subjected to a JAM assay. Four out of seven patients had a detectable CTL response subsequent to the third DC vaccination. A robust infiltration with CD8+ and CD45RO+ T cells was found in two of four patients who underwent re-resection for tumor recurrence, which was not apparent in the same patient's tumor specimen before receiving the DC vaccinations. Long-term survival data of vaccine study group was compared to similar age- and gender-matched controls that underwent surgical resection after external beam radiotherapy. On comparison, the median survival was higher in the study group compared with the control population, 455 and 257 days, respectively. Therefore, an inference can be made that DC vaccination may generate a survival benefit. The study data also demonstrated that DC vaccination was effective in generating anti-tumor immune responses as shown by peripheral cytotoxicity assays and intratumoral T cell infiltration. In addition, DC vaccination was determined to be safe, as described in the data. There has been no report from past and ongoing DC trials of Grade III or Grade IV NCI common toxicity criteria (CTC) adverse events associated with DC vaccinations. The safety, effectiveness, and survival benefits of DC vaccinations have led to the expansion of this study into Phase II clinical trials at Cedars-Sinai Medical Center.

As described above, current strategies using DCs in the treatment of brain tumors are based on loading these cells with tumor-derived proteins ex vivo. Sufficient loading requires surgical resection of ample amount of tumor to serve as an antigenic source for DC priming. Therefore, malignant tumors in eloquent areas that are deemed surgically unresectable limit the applicability of current DC vaccination paradigms. However, recent evidence has described that actual physical interaction between DCs and tumor cells may be necessary for proper induction of effective therapeutic immunity.[11] Recent data has also shown that DCs are capable of processing apoptotic tumor cells to induce CTL activity.[3,4]

To enhance glioma specific antigen presentation to the immune system in vivo, Yu et. al. proposed the novel approach of administering DCs directly into tumor after a course of stereotactic radiotherapy to induce apoptosis. Using this approach would circumvent the prerequisite of surgically acquiring sufficient amount of tumor for making the vaccine and the process of DC pulsing with tumor antigen ex vivo before vaccination. In light of this, investigators designed and tested a novel therapy involving inoculation of immature bone marrow-derived DC directly into established intracranial gliomas in rats.[14] To provide an appropriate antigenic source for DC priming, implanted gliomas were partially

irradiated to induce apoptosis. After intracranial inoculation, DCs drained to deep cervical lymph nodes and elicited systemic anti-tumor cytotoxic immunity. When compared with monocyte-treated controls, a robust intratumoral T cell infiltration, inhibition of glioma growth, and a significant prolongation in survival was found. As demonstrated by the aforementioned results, an effective method of stimulating anti-tumor immunity, leading to tumor rejection and immune memory, is by directly placing immature DCs in contact with partially apoptotic tumor. Radiotherapy, along with this strategy, may be of particular benefit. In fact, Kikuchi et al.[22] found encouraging results in a similar intracranial DC vaccination strategy that involved inoculation of immature DCs into partially irradiated intracranial gliomas. Akasaki et al.[1] vaccinated DC-glioma fusion cells in intracranial glioma bearing mice as part of a strategy to improve DC mediated tumor antigen presentation by means of enhancing tumor cell-APC interaction. Their results demonstrated that this strategy significantly inhibited intracranial glioma growth, improved survival in treated animals and strongly increased specific anti-timor CTL activity. Kikuchi et al.[23] later used this strategy in a Phase I clinical trial. A series of between three and seven peripheral intradermal vaccinations of DC-autologous glioma fusion cells was administered to a total of eight patients (five with glioblastoma multiforme, two with Grade III astrocytoma, and one with anaplastic oligodendroglioma). The results did not demonstrate significant increase in tumor specific CTL activity, and only very small and temporary responses to therapy were detected in two patients who later developed progressive disease.

The disappointing results of their Phase I clinical trial were speculated by Kikuchi et al.[23] to be attributed to the in vitro glioma culture process, which, during that time, may have changed the profile of tumor expressed immunogenic antigens, thereby rendering their fusion vaccines ineffective against the residual primary or recurrent tumor cells in vivo. In their earlier preclinical study, this was not a factor.[1] Their preclinical study used a uniform immortalized glioma cell line that was not subject to the same degree of in vitro selection pressures as the primary glioma explants used in their clinical trial. Systemic and intracranial T-cell responses modulated by the local central nervous system tumor microenvironment induced by DC vaccination in glioblastoma patients was recently reported by Liau et al.[28]

A DC vaccine pulsed ex vivo with fresh whole tumor cell lysate was another described clinical trial using DC pulsed with autologous tumor lysate. This study involved fourteen patients, 12 of whom had recurrent disease (nine with GBM and three with AA), whereas two were newly diagnosed (one each with GBM and AA). Yet to be identified, an optimal source of immunogenic tumor antigens was hypothesized to be tumor lysate. A significant cytotoxic response against tumor in six out of 10 patients was demonstrated after vaccination with tumor lysate primed DCs, as determined by qPCR analysis of IFNγ message in restimulated PBMC,[54] clearly demonstrating that DC therapy generates potent peripheral anti-tumor cytotoxic immunity. In a subset of glioma patients treated with DC vaccinations, the presence of MHC class I-restricted CTL recognizing the tumor associated antigen TRP-2, HER-2, MAGE-1, and gp100 in PBMC was demonstrated, using HLA restricted tetramer staining,[38] to be significantly increased after treatment (results from one representative patient are depicted in *Figure 41.1*).[30] A powerful tool to monitor the efficacy of generating antigen-specific responses is the utilization of HER-2, MAGE-1, and gp100, TRP-2 and AIM-2 as markers.[2,29–31,54,55]

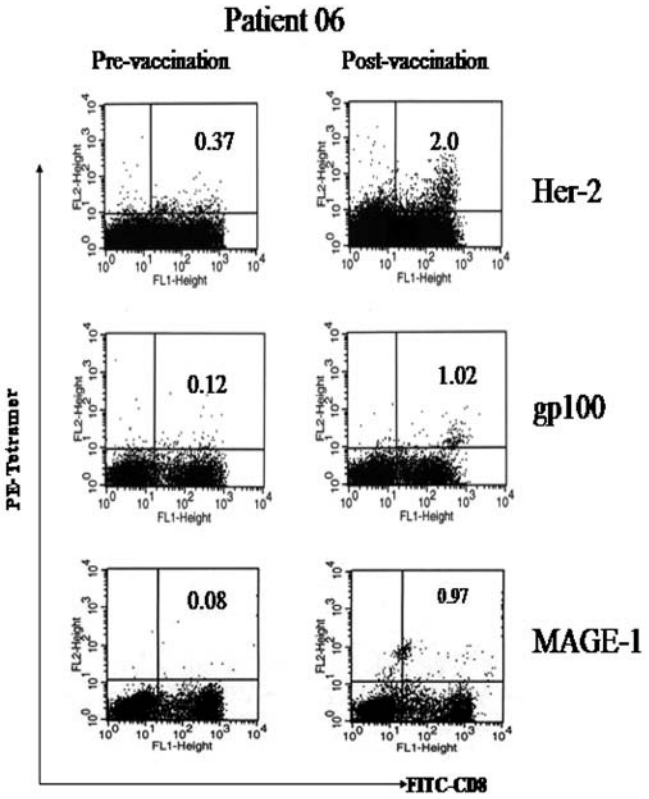

FIGURE 41.1. Representative flow cytometry plots from a single glioma patient vaccinated with autologous tumor lysate pulsed DCs. PBMC isolated pre- (left column) and post-vaccination (right column) were stained with HLA restricted tetramers for HER-2, gp100, and MAGE-1 (y-axis). Additionally, cells were stained for the CD8 antigen (x-axis). Plots indicate a significant increase in the number of cells that registered as double positive (i.e. bound to antigen specific tetramers and positive for CD8). This demonstrates an expansion in the populations of CTL specific for these TAAs in this patient after DC vaccination.

SENSITIZATION OF GLIOMA CELLS TO CHEMOTHERAPY AFTER DENDRITIC CELL THERAPY

In 1998, Firk[16] found that, after tumor cells were co-cultured with CTLs to eliminate tumor cells expressing higher levels of MHC-I and relevant tumor antigen, CTL-resistant tumor cells exhibited increased drug sensitivity. Liu et al. recently found significant drug resistance to carboplatin and temozolomide compared to wild type U-373 (W-U373) resulted from the TRP-2 transfected cell line (TRP-2-U373). CTL-resistant tumor cells (IS-TRP-2–373) developed significant increased sensitivity to carboplatin and temozolomide, compared with W-U373, after immunoselection by TRP-2 specific CTL clone.

After active immunotherapy against unselected glioma antigens using tumor lysate-loaded DCs in our Phase I DC vaccination clinical trial, TRP-2-specific cytotoxic T cell activity was detected in patients' PBMC.[30] Tumor cell specimens were taken from post-vaccination resections from two patients who developed CTL to TRP-2. Compared with autologous cell lines derived from pre-vaccination resections in two patients who demonstrated CTL response to TRP-2 (*Fig. 41.2*), these specimens demonstrated significantly lower TRP-2 expression (*Fig. 41.3*) and higher drug sensitivity to carboplatin and temozolomide. Given this finding, targeting TRP-2 may provide a new strategy in improving chemotherapy sensitivity. However, all forms of drug resistance in tumor cells do not seem to develop with TRP-2. One can, therefore, speculate that other drug resistance-related proteins, such as EGFR, MDR-1, MRPs, HER-2, and survivin, may also decrease after DC vaccination. In unpublished data, CJ Wheeler found that EGFR expression was specifically decreased after vaccination in glioma patients. Given this, determining whether or not immune responses to other drug resistance modifiers can similarly influence chemotherapeutic efficacy in human cancer will be important.

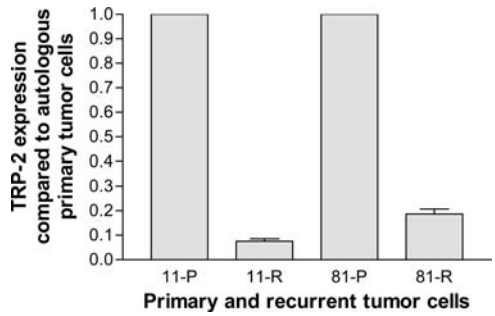

FIGURE 41.3 TRP-2 expression in primary (P) and recurrent (R) tumor cells. Total RNA was extracted from tumor cells derived from patient No. 81 and patient No. 11. TRP-2 mRNA expression was measured by real-time qPCR. The expression was firstly normalized by internal control B-actin. The relative TRP-2 mRNA level of recurrent tumor was presented as the fold decrease compared to autologous primary tumor cells. (from, Liu G. et al.: Cytotoxic T cell targeting of TRP-2 sensitizes human malignant glioma to chemotherapy. **Oncogene** 24: 5226–5234, 2005).

Loss of chromosomal arms 1p and 19 q is another mechanism that may contribute to the sensitization of tumor cells to chemotherapy after vaccination. A unique constellation of molecular changes have been identified in previous studies, including allelic loss of chromosome 1p and coincidental loss of chromosomal arms 1p and 19q (frequency, 50–70%), which, in some gliomas, particularly in anaplastic

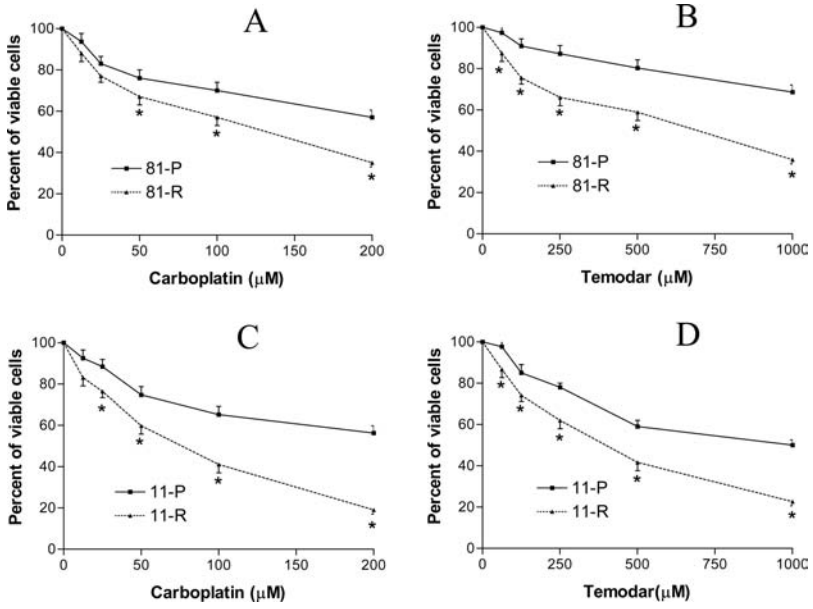

FIGURE 41.2. Drug sensitivity of in primary (P) and recurrent (R) tumor cells. Tumor cells derived from patient No. 81 and patient No. 11 were treated with various concentrations of (*A* and *B*) carboplatin; (*C* and *D*) temozolomide for 48 hours. Asterisk indicates $P < 0.05$ compared with autologous primary tumor cells. Data are from three independent experiments. (from, Liu G. et al.: Cytotoxic T cell targeting of TRP-2 sensitizes human malignant glioma to chemotherapy. **Oncogene** 24: 5226–5234, 2005).

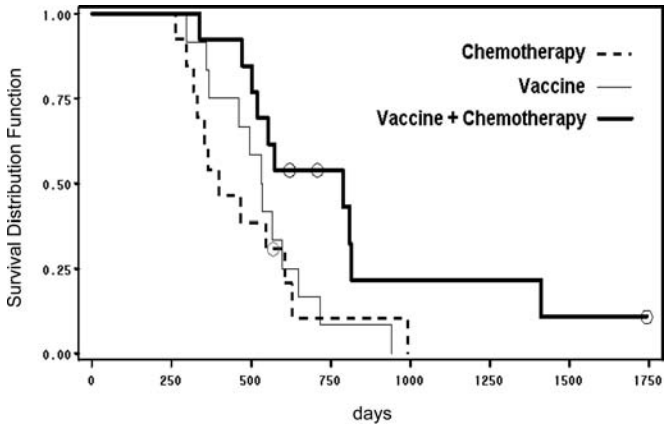

FIGURE 41.4. Overall survival in vaccine, chemotherapy, and vaccine+ chemotherapy groups. Overall survival was defined as the time from first diagnosis of brain tumor (de novo GBM in all cases) to death due to tumor progression. Kaplan-Meier survival plots with censored values in *open circles* are shown for each group. Survival of the vaccine group was identical to that of chemotherapy group ($P = 0.7$, log-rank test). Survival of vaccine + chemotherapy group was significantly greater relative to survival in the other two groups together ($P = 0.048$, log-rank test), greater than survival in the chemotherapy group alone ($P = 0.028$, log-rank test), and greater than survival in the vaccine group alone ($P = 0.048$, log-rank test). Two of the three patients exhibiting objective tumor regression survived for more than 2 years (730 d) after diagnosis. (from, Wheeler CJ, et al.: Clinical responsiveness of glioblastoma multiforme to chemotherapy after vaccination. **Clin Cancer Res** 10:5316–5326, 2004).

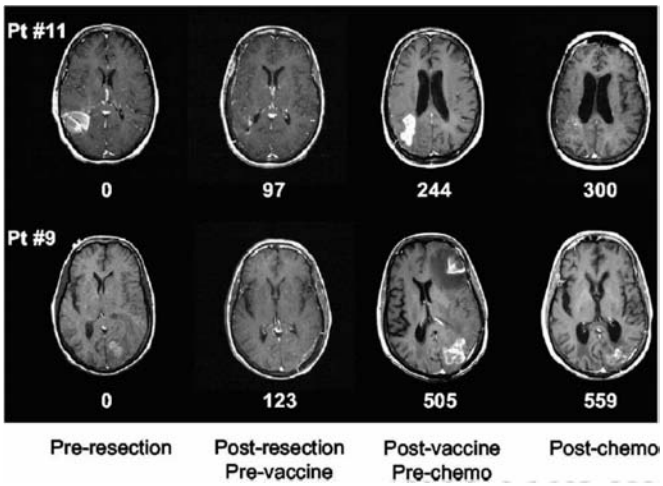

FIGURE 41.5 Tumor regression following post-vaccine chemotherapy. Relative days after diagnosis are represented by the *numbers* under individual MRI scans, with individual patient scans in each row. Patient 11 recurred 82 days after vaccine initiation; Patient 9 recurred 147 days after vaccine initiation, was treated surgically, and recurred 227 additional days (374 d total) after vaccine initiation. (from, Wheeler CJ, et al.: Clinical responsiveness of glioblastoma multiforme to chemotherapy after vaccination. **Clin Cancer Res** 10:5316–5326, 2004).

and non-anaplastic oligodendroglioma, strongly predicts a far greater likelihood of chemotherapeutic response.[7,10] In a series of 55 Grade II and III oligodendrogliomas, for example, the principal independent predictor of progression-free survival after chemotherapy with procarbazine, lomustine, and vincristine plus radiotherapy was loss of heterozygosity of chromosome 1p; the median progression-free survival for 19 patients whose tumors retained both copies of 1p was only 6 months compared with 36 patients whose tumors had lost 1p alleles was 55 months.[7] Specific molecular genomic changes may prove useful as markers of relative chemosensitivity in a subset of high-grade gliomas, particularly in anaplastic oligodendrogliomas. Loss of heterozygosity (LOH) at the chromosomal loci, as previously described using polymorphic micro-satellite markers, of tumor DNA from laser-dissected pre- and post-vaccine pathological specimens was analyzed.[37] This analysis revealed that after DC vaccination of young (responsive, <55 yr) patients, a prominent change in allelic loss frequency was localized to chromosomal region 1p36; 100% of patients' tumors exhibited 1p36 LOH after vaccination, whereas only 33% of patient's tumor exhibited 1p36 LOH before vaccination (n = 6) (Wheeler CJ, unpublished data). The potential of improving chemosensitivity of GBMs by using DC active immunotherapy to elicit fundamental physiological changes have been demonstrated in current studies.

CLINICAL RESPONSIVENESS OF GLIOBLASTOMA MULTIFORME TO CHEMOTHERAPY AFTER VACCINATION

A novel approach for the treatment of recurrent malignant gliomas involves the use of cancer vaccines.[52] Two excellent reviews have recently described the clinical and immunologic outcome of these vaccination studies in cancer patients.[15,42] There is controversy surrounding the clinical efficacy of therapeutic cancer vaccines for the treatment of any human tumor, as tumor destruction and/or extended survival have not been consistently observed in cancer patients who have received vaccinations.[17,26,43,54,110] The use of passive, adoptive, and non-specific strategies yielded limited benefits in previous immunotherapeutic treatments for gliomas[56]; such treatments involved intrathecal or intratumoral administration of autologous lymphocytes, interleukin 2 (IL-2) and lymphokine-activated killer (LAK) cells, and interferons.[24,36,48,49] The non-specific immune response that those approaches generated was likely due to the absence of a significant anti-tumor effect. Presently, DC cancer vaccines, in most patients, reliably elicit tumor-reactive cytotoxic T lymphocytes (CTL).[8,26,41] In a subset of patients with glioblastoma, DC vaccination have been shown to induce a

cytotoxic T cell response to autologous tumor and specific tumor associated antigens.[2,29,30,51,54,55] Induction of cytotoxic memory T cells to localize in intracranial tumor in a subset of patients was also demonstrated.[54,55] It is unclear why tumor recurs despite CTL induction by DC vaccination, however, the processes of immunoselection and immunediting, which allows tumor cells to escape from CTLs by antigen loss, is one possibility.[13,21] The potential synergies between immunotherapy and other therapies must, therefore, be investigated due to the clinical inconsistency of cancer vaccines and the effects of immunoselection on tumor evolution.[34,39,51]

Clinical trials conducted at Cedars-Sinai Medical Center[51] and Brigham and Women's Hospital[18] examine the synergy of vaccines with chemotherapy treatment. A retrospective analysis of clinical outcomes (survival and progression times) in 25 vaccinated (13 with and 12 without subsequent chemotherapy) and 13 non-vaccinated de novo glioblastoma (GBM) patients receiving chemotherapy was performed. Longer survival times and significantly longer times to tumor recurrence after chemotherapy relative to their own previous recurrence times, as well as to patients receiving vaccine or chemotherapy alone, was demonstrated in patients who received post-vaccine chemotherapy (*Fig. 41.4*). A dramatic response was demonstrated in two of these patients who underwent treatment with temozolomide after recurrence (*Fig. 41.5*). Therapeutic DC vaccination works in synergy with subsequent chemotherapy to elicit tangible clinical benefits for GBM patients mediated by sensitizing tumor cells to therapeutic drugs after CTL immune selecting to deplete drug resistant tumor cells. This is based on the evidence that DC vaccinations induce specific CTL targeting the drug resistance-related tumor-associated antigens and clinical observations. These clinical trials strongly support the concept for the use of immunotherapy to sensitize tumor cells in chemotherapy.

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