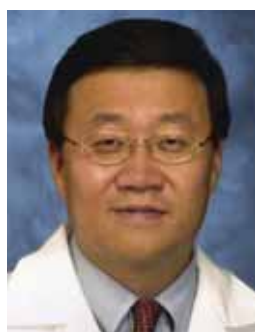


For reprint orders, please contact [reprints@future-drugs.com](mailto:reprints@future-drugs.com)



**Gentao Liu and John S Yu<sup>†</sup>**  
<sup>†</sup>Author for correspondence  
 Maxine Dunitz Neurosurgical  
 Institute, Cedars-Sinai Medical Center,  
 CA 90048, USA; Division of  
 Hematology/Oncology, Cedars-Sinai  
 Medical Center/David Geffen School of  
 Medicine at UCLA, CA 90048, USA  
 Tel.: +1 310 423 0845  
 Fax: +1 310 423 0810  
[yuj@cshs.org](mailto:yuj@cshs.org)

## Cancer vaccines: a novel strategy to sensitize malignant glioma to chemotherapy

'...the direct combination of immunotherapy with chemotherapy might open a new avenue in cancer treatment and show ... how cancer immunotherapy can be effectively used in patients with solid tumors.'

*Expert Rev. Neurotherapeutics* 7(10), 1235–1237 (2007)

Drug resistance represents a major cause of chemotherapy failure in patients with cancer, including malignant glioma. Accumulating evidence indicates that immunotherapy may represent an effective approach to overcome the problem.

Drug resistance, intrinsic or acquired, is a problem for all chemotherapeutic agents. As the mechanisms responsible for drug resistance become clearer, several strategies have been tested or proposed to overcome drug resistance and improve clinical outcome. Much of the past three decades has been spent on developing new cytotoxic drugs and exploring combination chemotherapy regimens that would improve clinical outcome.

Unfortunately, in most cases, at least partial cross-resistance exists among the currently available cytotoxic drugs. Although gene therapy and chemosignal therapy in combination with chemotherapy are found to be very promising treatments to modulate response to chemotherapy, the genetic heterogeneity of glioma cells and the multifactorial nature of drug resistance significantly limit the efficacy of these strategies.

An ideal strategy would be one whereby multiple pathways of drug resistance are targeted by immunotherapy [1].

Immunization with cancer vaccines in particular offers advantages that other cancer therapy strategies do not. First, it is highly specific for cancer cells and, therefore, low toxicity should be expected. Second, it recognizes and eliminates cancer cells regardless of their phase in the cell cycle. Third, tumors that developed drug resistance would still be a suitable target for immunotherapy. Fourth, vaccination offers the unique potential for a durable antitumor effect owing to the phenomenon of immunologic memory, potentially obviating the need for prolonged, repetitive cycles of therapy.

Fifth, immunotherapy offers the possibility of preventative immunization of high-risk patients [2].

The challenge in developing therapeutic cancer vaccines to induce tumor specific T-cell responses has been in identifying and successfully presenting immunogenically relevant tumor antigens to T cells *in vivo*.

The challenge in developing therapeutic cancer vaccines to induce tumor specific T-cell responses has been in identifying and successfully presenting immunogenically relevant tumor antigens to T cells *in vivo*. The antigenic peptides of these gene products, which can be recognized by cytotoxic T lymphocytes (CTLs) in an MHC class I restricted manner, have also been identified, and some of them have been used in peptide-based cancer vaccines in clinical trials for cancer patients. Recently, TRP-2, HER-2, gp100 and MAGE-1 were identified as immunotherapeutic targets in

glioma. Most importantly, a number of tumor-associated antigens, such as TRP-2, MEGE-1, HER-2, EGFR, MRP-3, EphA2, thymidylate synthase and the inhibitor of apoptosis protein (survivin), are not only T-cell targets, but also functionally mediate drug resistance. Some tumor antigens, although neither exclusive for nor specific to brain tumors, have been associated with the formation of a chemoresistant phenotype. Tumor-associated antigens can mediate resistance to chemotherapeutic agents in glioma. These tumor antigens may serve as a powerful link between chemotherapy and immunotherapy. Specific targeting of these tumor antigens by a cancer vaccine provides a novel strategy to overcome drug resistance of tumor cells [1].

Dendritic cells (DCs) are the most potent antigen-presenting cells of the immune system and have been shown to stimulate antibody and cell-mediated immune responses against tumor-associated antigens. *Ex vivo*-generated and tumor antigen-loaded DCs have been successfully introduced in clinical vaccination protocols, which have proven to be feasible and effective in some glioma patients. We have shown that DC vaccination induces a cytotoxic T-cell response to autologous tumor and specific tumor-associated antigens in a subset of patients with glioblastoma [3,4]. DC vaccination also induces cytotoxic memory T cells to localize in intracranial tumor in a subset of patients [3,4]. Recently, Liau and colleagues reported that DC vaccination in glioblastoma patients induces systemic and intracranial T-cell responses modulated by the local CNS tumor microenvironment [5].

In another study, tumor cells were derived from post-vaccination resections from two patients who developed CTLs to TRP-2. These specimens demonstrated significantly lower TRP-2 expression and higher drug sensitivity to carboplatin and temozolomide than those autologous cell lines derived from pre-vaccination resections in two patients who demonstrated CTL response to TRP-2 [6]. Therefore, it is reasonable to speculate that other drug resistance-related proteins, such as EGFR, MDR-1, MRP-3, HER-2 and survivin, might also decrease after vaccination. Another mechanism may contribute to the sensitization of tumor cells to chemotherapy derived from the loss of chromosomal arms 1p and 19q after vaccination. Current studies demonstrated the potential of current DC active immunotherapy to elicit fundamental physiological changes in glioblastoma tumors, thereby improving sensitivity to chemotherapy. Dramatic clinical responsiveness of glioblastoma multiforme to chemotherapy after vaccination was observed in our clinical trials. Clinical outcomes (survival and progression times) were analyzed retrospectively in 25 vaccinated (13 with and 12 without subsequent chemotherapy) and 13 nonvaccinated *de novo* glioblastoma patients receiving chemotherapy. Patients receiving post-vaccine chemotherapy had longer survival times and exhibited 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. Most importantly, two of these patients underwent treatment with temozolomide after recurrence and responded dramatically [7]. Based on the evidence

that DC vaccination induces specific CTL targeting drug resistance-related tumor-associated antigens and our clinical observations, we believe that therapeutic vaccination works in synergy with subsequent chemotherapy to elicit tangible clinical benefits for glioblastoma patients mediated by sensitizing tumor cells to therapeutic drugs after CTLs specifically deplete drug-resistant tumor cells. What is more, very similar results were reported by several other groups. Gribben and colleagues immunized 17 patients with different types of cancer with antigen cytochrome P450 1B1 (CYP1B1) [8]. In ten of 11 patients who failed to develop immunity to CYP1B1, the disease progressed and did not respond to salvage chemotherapy or radiotherapy. Conversely, five of six patients who did develop immunity to CYP1B1, and needed salvage treatment for progressive metastatic disease, showed a substantial clinical response to chemotherapy, which lasted longer than 1 year in most patients. In another study, Gabrilovich and colleagues treated 29 patients with extensive stage small-cell lung cancer with a p53-based DC vaccine [9]. The cancer progressed after vaccination in 23 patients who were then treated with salvage chemotherapy (paclitaxel or carboplatin regimens). The clinical response rate was 61.5%, and 11 (38.1%) patients were alive 1 year after vaccination. A direct positive correlation between immune response to vaccination and clinical response to chemotherapy was noted.

Furthermore, Arlen and colleagues treated 28 patients who had metastatic prostate cancer with prostate-specific antigen vaccine [10]. Patients were randomized to receive either vaccine and weekly docetaxel or vaccine alone. A total of 11 patients on vaccine alone whose disease progressed crossed over to receive docetaxel at the time of progression.

Median progression-free survival on docetaxel was 6.1 months after receiving the vaccine compared with 3.7 months with the same regimen in a historical control. In a review of five prostate cancer vaccine trials, National Cancer Institute researchers offer evidence that patients who receive vaccines may respond better to subsequent chemotherapy or hormone treatment [11]. These clinical trials strongly support the concept of utilization of immunotherapy to sensitize tumor cells in chemotherapy.

Success with DC immunotherapy to sensitize tumor cells to chemotherapy is dependent upon the induction of robust immunity against drug resistance-related tumor antigens. A number of tumor-associated antigens related to drug resistance have been identified, and several have been used to generate vaccines used in clinical trials. It is not yet clear, however, which chemosensitizing antigens will be most effective in inducing antitumor immunity. Tumor immunity may require vaccination with multiple target antigens. Furthermore, it may be critical to target antigens that are essential for cancer cell survival and that are expressed on most cancer cells. Recently, we and other groups have identified a small population of cancer stem cells in adult and pediatric brain tumors [12,13]. Cancer stem cells have been demonstrated to be resistant to chemotherapy [14] and radiation therapy [15]. Therefore, functional genomics and

'Drug resistance represents a major cause of chemotherapy failure in patients with cancer, including malignant glioma.'

proteomics will have implications on the field of tumor antigen discovery owing to the possibility of molecular characterization of whole transcriptomes and proteomes of cancer stem cells, thereby also identifying potential new targets on cancer stem cells for immunotherapy to sensitize chemotherapy.

The fact that these studies were small and were not originally designed to test this concept and are therefore prone to bias needs to be emphasized. This novel hypothesis needs to be tested in randomized controlled clinical trials on the basis of an intent-to-treat analysis. It would be important to establish the optimum timing and scheduling of immunotherapy and chemotherapy, to identify whether this effect is limited to a specific type of chemotherapy and whether immunotherapy can also augment the clinical effect of radiotherapy [16].

Although this novel concept is far from being firmly established, the data is probably sufficient to generate cautious optimism that the direct combination of immunotherapy with

chemotherapy might open a new avenue in cancer treatment and show, for the first time, how cancer immunotherapy can be effectively used in patients with solid tumors. It is possible that, in the end, paradoxical use of immunotherapy in direct combination with chemotherapy in patients with cancer might become a viable option for the treatment of patients with advanced-stage cancer [16].

#### Financial & competing interests disclosure

*The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.*

*No writing assistance was utilized in the production of this manuscript.*

#### References

- Liu G, Black KL, Yu JS. Sensitization of malignant glioma to chemotherapy through dendritic cell vaccination. *Expert Rev. Vaccines* 5(2), 233–247 (2006).
- Emens LA, Jaffee EM. Toward a breast cancer vaccine: work in progress. *Oncology* 17(9), 1200–1211; discussion 1214, 1217–1208 (2003).
- Yu JS, Wheeler CJ, Zeltzer PM *et al.* Vaccination of malignant glioma patients with peptide-pulsed dendritic cells elicits systemic cytotoxicity and intracranial T-cell infiltration. *Cancer Res* 61(3), 842–847 (2001).
- Yu JS, Liu G, Ying H, Yong WH, Black KL, Wheeler CJ. Vaccination with tumor lysate-pulsed dendritic cells elicits antigen-specific, cytotoxic T-cells in patients with malignant glioma. *Cancer Res* 64(14), 4973–4979 (2004).
- Liau LM, Prins RM, Kiertscher SM *et al.* Dendritic cell vaccination in glioblastoma patients induces systemic and intracranial T-cell responses modulated by the local central nervous system tumor microenvironment. *Clin. Cancer Res* 11(15), 5515–5525 (2005).
- Liu G, Akasaki Y, Khong HT *et al.* Cytotoxic T cell targeting of TRP-2 sensitizes human malignant glioma to chemotherapy. *Oncogene* 24(33), 5226–5234 (2005).
- Wheeler CJ, Das A, Liu G, Yu JS, Black KL. Clinical responsiveness of glioblastoma multiforme to chemotherapy after vaccination. *Clin. Cancer Res* 10(16), 5316–5326 (2004).
- Gribben JG, Ryan DP, Boyajian R *et al.* Unexpected association between induction of immunity to the universal tumor antigen CYP1B1 and response to next therapy. *Clin. Cancer Res* 11(12), 4430–4436 (2005).
- Antonia SJ, Mirza N, Fricke I *et al.* Combination of p53 cancer vaccine with chemotherapy in patients with extensive stage small cell lung cancer. *Clin. Cancer Res* 12(3 Pt 1), 878–887 (2006).
- Arlen PM, Gulley JL, Parker C *et al.* A randomized Phase II study of concurrent docetaxel plus vaccine versus vaccine alone in metastatic androgen-independent prostate cancer. *Clin. Cancer Res* 12(4), 1260–1269 (2006).
- Schlom J, Arlen PM, Gulley JL. Cancer vaccines: moving beyond current paradigms. *Clin. Cancer Res* 13(13), 3776–3782 (2007).
- Singh SK, Hawkins C, Clarke ID *et al.* Identification of human brain tumour initiating cells. *Nature* 432(7015), 396–401 (2004).
- Yuan X, Curtin J, Xiong Y *et al.* Isolation of cancer stem cells from adult glioblastoma multiforme. *Oncogene* 23(58), 9392–9400 (2004).
- Liu G, Yuan X, Zeng Z *et al.* Analysis of gene expression and chemoresistance of CD133<sup>+</sup> cancer stem cells in glioblastoma. *Mol. Cancer* 5, 67 (2006).
- Bao S, Wu Q, McLendon RE *et al.* Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature* 444(7120), 756–760 (2006).
- Gabrilovich DI. Combination of chemotherapy and immunotherapy for cancer: a paradigm revisited. *Lancet Oncol.* 8(1), 2–3 (2007).

#### Affiliations

- Gentao Liu, PhD  
Maxine Dunitz Neurosurgical Institute,  
Cedars-Sinai Medical Center, CA 90048, USA;  
Division of Hematology/Oncology, Cedars-Sinai  
Medical Center/David Geffen, School of  
Medicine at UCLA, CA 90048, USA  
Tel.: +1 310 423 7632  
Fax: +1 310 423 7588  
liug@ucla.edu
- John S Yu, MD  
Maxine Dunitz Neurosurgical Institute,  
Cedars-Sinai Medical Center, CA 90048, USA;  
Division of Hematology/Oncology, Cedars-Sinai  
Medical Center/David Geffen School of  
Medicine at UCLA, CA 90048, USA  
Tel.: +1 310 423 0845  
Fax: +1 310 423 0810  
yuj@cshs.org