Published in final edited form as:

Am Soc Clin Oncol Educ Book. 2014 ; : 42–50. doi:10.14694/EdBook_AM.2014.34.42.

The Value of EGFRvIII as the Target for Glioma Vaccines

Pedro R. Lowenstein, MD, PhD and Maria G. Castro, PhD

Department of Neurosurgery and Cell and Developmental Biology, Graduate Program in Immunology, and Graduate Program in Cancer Biology, The University of Michigan Comprehensive Cancer Center, The University of Michigan School of Medicine, Ann Arbor, MI

OVERVIEW

Malignant brain tumors continue to be rapidly progressive and resistant to most treatments. Even with state-of-the-art standard of care (surgery, chemotherapy, and radiotherapy) long-term survival in the last 80 years improved from 6 to 15 months. Improved imaging has also likely contributed to prolonged survival. Immunotherapy for cancer dates back to publications from 1742. The central idea is that the immune system can detect and eliminate foreign antigens, either from infectious agents or tumors, and thus could be therapeutic in brain tumors. Recent introduction of immune modulators of cytotoxic T-lymphocyte antigen (CTLA)-4 and programmed cell death 1/programmed cell death 1 ligand (PD-1/PDL1) add much excitement to this field. For brain tumors, there are several ongoing phase I and III trials to determine whether any of the current immunotherapy approaches can demonstrate activity in randomized, controlled double-blinded trials—with ongoing and historical trials presented in tables within the manuscript.

Immunotherapy has explored the use of various types of antigens (obtained either from homogenates of patients' tumors or synthetically produced), and various immunization procedures and adjuvants. Glioma antigens have also been isolated from the patients' own tumor, then produced in vitro (for example the glioma antigen EGFRvIII), and used to immunize patients directly, or with carriers such as dendritic cells with or without additional adjuvants. Several of these practical approaches are currently in phase III trials. Remaining challenges are how to increase the percentage of complete responses and response duration, and the enigmatic absence of an almost total lack of adverse brain inflammation following immunization of brain tumor patients, as has been observed following immunization against brain antigens in other diseases, such as Alzheimer's Disease.

Recent improvements in the treatment of high-grade glioma tumors (glioblastoma multiforme, WHO grade 4) have been mostly credited to improvements in standard therapies. These improvements include surgery with electro-physiological monitoring that allows resection of the tumor with reduced negative neurological outcomes, optimization of radiotherapy, and the timing of delivery of temozolomide, which has allowed an increase in

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Corresponding author: Pedro R. Lowenstein, MD, PhD, Department of Neurosurgery, Department of Cell and Developmental Biology, University of Michigan School of Medicine, MSRB II, Room 4570, 1150 West Medical Center Dr., Ann Arbor, MI 48109; pedrol@umich.edu.

Disclosures of Potential Conflicts of Interest

The author(s) indicated no potential conflicts of interest.

patient survival, although that still depends on the particular institution providing care. In the highest complexity centers with therapy optimization a median survival of 16 months is now considered standard, with patients occasionally surviving longer than 2 years. Nevertheless, even with optimized use of temozolomide, gross total resection assessed by MRI, and radiotherapy, routine long-term survival of several years remains elusive in the majority of cases.¹

There are several new therapies waiting in the wings. Gene therapy²⁻⁵ has been moving new therapeutic approaches into large phase III trials, myriads of new chemotherapeutics targeted to potentially driving mutations in glioblastoma are progressing at breakneck speed, and new drugs that stimulate the immune response such as antibodies CTLA-4 and PD1 are being tested.⁶⁻⁸ Despite the frantic translational activity no new treatments, with the possible exception of one anti-angiogenic medication (bevacizumab) integrated into standard of care for patients with recurrent glioblastoma, have yet been incorporated as standard adjuncts to the standard of care triad.⁹⁻¹¹

Different immunotherapeutic approaches have been tested preclinically and are currently in evaluation in early phase I to phase III trials. Approaches vary from the use of autologous dendritic cells loaded with tumor peptides removed from the patients' own tumors, to autologous dendritic cells loaded with synthesized tumor antigenic peptides, to the use of autologous and/or allogeneic T cells engineered or selected to recognize glioma antigens. Dendritic cells are usually given as intradermal or subcutaneous vaccines, where as T cells are either delivered systemically or into the postsurgical resection cavity.

Overall increased survival benefit obtained so far has not been universal.^{12,13} Even if individual trials routinely report significant extensions of life, none of these novel therapies has yet been incorporated to the standard of care armamentarium. Importantly, although there is a potential to stimulate brain autoimmune responses as normal brain antigens are likely to be present among the peptides used to load dendritic cells, no such autoimmune responses or brain inflammation has been detected in most trials. An absence of autoimmune side effects has usually been taken to indicate the safety of the different kinds of dendritic cell vaccination approaches. On the other hand, there is a possibility that the almost complete lack of side effects could actually be a reflection of inefficient immunization in patients suffering from high-grade glioma, a population of patients already known to display pre-existing systemic immune-suppression. In comparison, when patients with Alzheimer's were immunized systemically against beta-amyloid peptides, 10% to 15% developed clinically significant brain inflammation, which led to a hold on ongoing trials. As systemic immunization against brain antigens ought to lead to significant brain autoimmunity in at least a percentage of patients, the absence of such responses from glioma immunotherapy trials requires further examination. Of interest, there is a single report of brain inflammation after immunization with dendritic cell vaccinations.¹⁴ In that report of two subjects (pet dogs with high-grade glioma and treated experimentally at the University of Minnesota Veterinary School), the vaccines' potency was attempted to be increased through concomitant delivery of interferon-gamma. The life-threatening brain inflammation may indicate that dendritic cell vaccines require additional boosts with other immune-stimulators. Similar brain inflammation has been reported in some patients with melanoma metastasis to

the brain and treated with inhibitors of CTLA4-Ig.^{15–17} Taken together these results indicate that brain inflammation that followed may be a side effect that could indicate potency of immune responses in attacking antigenic cells in the brain. Additionally, we have recently shown that *Ad-TK* and *Ad-Flt3L* gene therapy¹⁸ and dendritic cell vaccination potentiate each other.¹⁹ In a rat glioma model, when dendritic cell vaccination was administered at 10 to 14 days post-tumor implantation there were no effects on glioma growth, whereas administration of *Ad-TK* and *Ad-Flt3L* gene therapy provided a 50% rate of long-term survival. When administered jointly, animal survival was close to 90%.¹⁹ We interpret these data as indicating that dendritic cell vaccination may need immunologic adjuvants to exert its full effects. Which mechanisms normally inhibit the full efficiency of dendritic cell vaccination and which can be overcome by *Ad-TK* and *Ad-Flt3L* gene therapy, or delivery of interferon-gamma, deserves further study.

A variety of antigens specifically enriched in brain tumors have been proposed to serve as targets for immunotherapies (Tables 1 and 2). In addition, 25% to 30% of high-grade glioma tumors express a truncated variant of EGFR, known as EGFRvIII, which promotes ligand independent signaling. In-frame deletion of exons 2 through 7 from EGFR results in constitutively active kinase activity, and the presence of unique amino acid sequences at novel mutation induced junctions. The extracellular portion of EGFRvIII would make the tumor antigen more accessible to activated cytotoxic T cells. If EGFRvIII is indeed a driver of glioma tumors, directly attacking EGFRvIII and destroying glioma cells expressing EGFRvIII ought to provide a therapeutic benefit, as it would eliminate a powerful oncogenic stimulus. Paracrine interactions may also help multiply the effect of the mutation, or of the efforts to eliminate such cells from the tumors. Sampson et al have used vaccination against a 14-mer peptide, LEU-GLU-GLU-LYS-LYS-GLY-ASN-TYR-VAL-VAL-THR-ASP-HIS-CYS, derived from the mutated EGFR as antigen linked to KLH. Sampson et al have pioneered a number of early phase I and II trials. In earlier trials authors reported improved 6-month progression-free survival (PFS), and improved overall survival (OS); the median OS was 15 months in the contemporaneous control cohort compared with 26 months in the immunized cohort. The positive results have been used to support the progress to a randomized controlled double-blind phase III multicenter trial currently in progress.

In previous trials Sampson et al demonstrated increase in immunologic function specifically related to the recognition of the brain tumor antigen.²⁰ In patients with EGFRvIII-positive tumors at initial resection, immune responses could be stimulated in about 50% of patients. In trials of 17 immunized patients, six developed EGFRvIII antibodies and three developed evidence of a T cell response against EGFRvIII. Of potential clinical relevance, the development of either a humoral or a cellular response to mutated EGFRvIII appeared to provide increased OS, though the small numbers challenged in depth statistical analysis.^{21–23}

One of the most interesting pathophysiological studies performed by the authors was the study of EGFRvIII expression at tumor recurrence.²⁰ In 11 patients who could have tumor samples evaluated both before and after vaccination, 82% lacked EGFRvIII immunoreactivity at recurrence. These studies are a true tour de force as the difficulties involved are truly challenging. The authors favor the interpretation that this indicates tumor

escape, although at this stage it is impossible to differentiate tumor escape from loss of immunoreactivity because of the blocking of endogenous EGFRvIII immunoreactivity by the induced anti-EGFRvIII antibodies, or downregulation of EGFRvIII expression. Although the clinical progression of the disease would favor an interpretation of effective tumor control by the immunization followed by tumor mutation and escape, the absence of more detailed molecular studies and the low numbers involved preclude drawing definitive conclusions. In the future, analysis of comprehensive results from large-scale phase III, randomized, double-blind trials of patients with brain tumors immunoreactive for EGFRvIII will allow us to address the potential mechanisms of action of the EGFRvIII vaccine.

Even given the shortcomings of the data at this time, a discussion of potential mechanisms of action is warranted. An accurate understanding of the mechanisms of tumor response (and apparent escape from immunologic control) will be crucial in devising the future pathways of this therapeutic approach. Tumor heterogeneity,^{24,25} already classically detected in studies of tumor pathology—the classical name of the tumor is glioblastoma multiforme—has now been detected at the molecular level. Thus, even though 30% of patients may express mutated EGFRvIII, it is not yet known whether the mutation is present in all tumor cells, or only in a subset of cancer cells. If only present on a subpopulation of glioma cells, challenges in tumor sampling during biopsy could possibly explain changes in detected EGFRvIII expression; that is, even without changes in the population of tumor cells, unwitting biases in biopsy sampling may erroneously indicate changes in the expression profile of gliomas. Equally, selection could be at the level of expression, an outcome which will also indicate selection bias in the case of expression going below the limit of antigenic detection. Recently, it has also been described that amplified EGFR resides within abnormal mini-chromosomes that also participate in the selection of expressing cells when these are grown under selection.²⁶

If the observed selection does represent true elimination of the proportion of tumor cells expressing EGFRvIII, this would represent a strong indication that vaccination with multiple antigens may represent the way forward, as this would reduce the capacity of the tumors to escape from immune control. Therefore, detailed pathophysiological studies are necessary to understand both the power and the current challenges of immunization regimes against single antigens. A similar challenge was faced during the initial trials for the treatment of AIDS in patients infected with the rapidly mutating HIV virus. Initial treatment with only a single drug, rapidly selected mutant clones not expressing the drug target or cells with a mutated target now resistant to the drug inhibitor. However, as more anti-HIV drugs came onto the market it was realized that successful treatment necessitates simultaneous administration of multiple inhibitors of HIV's life cycle. Once three to five inhibitors are used simultaneously, viral replication is rapidly suppressed as the HIV genome is unable to find simultaneous selected mutants to three to five inhibitors. Thus, the treatment of HIV infection with multi-pronged attacks has led to the current increase in patient survival.²⁷

If we assume a similar pathophysiological process of selection and immune escape occurs on treatment of brain gliomas expressing EGFRvIII with specific vaccination paradigms, existing knowledge suggests that the combination of multiple inhibitors (or multiple vaccines directed against different antigens; or combination of immune-stimulators—e.g.,

antibodies to CTLA or PD-1-) would be predicted to eventually result in more effective results.

In vaccination against EGFRvIII it has been demonstrated that 33% of immunized patients developed humoral responses and 16% developed cellular responses.²⁰ Given that responses appear to involve the humoral and cellular arms of the adaptive immune response, it would be of importance to be able to determine the mechanism of action of each arm, as potentially both could have a positive therapeutic influence of disease outcome. Although in theory immunization aims to eliminate tumor growth by inducing the generation of antitumor cytotoxic T cells, antibodies against individual antigens could also provide therapeutic benefits. For example, antibodies binding to EGFRvIII on glioma cells may serve to kill tumor cells by antibody-dependent cell-mediated cytotoxicity, or by binding to EGFRvIII and inhibiting its signaling capacity by restricting its ability to diffuse across the plasma membrane. Eventual isolation of induced antibodies and a dissection in vitro of their mechanisms of action will be central to the future directions of glioma immunotherapy. Equally, it will be important to isolate antitumor T cells from those patients that mount such responses to study their function, pathogenicity, and capacity to kill glioma tumor cells in vitro. Potentially such T cells could also serve as sources to isolate antitumor T-cell receptor genes that could, at a later stage, be engineered into the bone marrow of patients within the context of the Chimeric Antigen Receptor (CAR) approach to engineering effective antitumor cytotoxic T cells.^{28,29}

Of course, outstanding questions to be resolved by future experiments remain: Why did only 50% of patients respond? Could this be a result of the general immune-suppression of glioma patients, or technical details of immunization procedures? Are the strongest results from this trial the increase in OS in a nonrandomized trial or the induction of immune responses in 50% of patients? It would be important to understand the mechanisms underlying a significant increase in OS even if immune responses were only detected in 50% of patients. Alternatively, the assessment of immune responses could be delayed until a treatment is found that provides a significant OS benefit in a randomized phase III trial, such as the sipuleucel-T trial for castration-resistant prostate cancer.³⁰ How are we to interpret that increased OS in the sipuleucel-T trial was seen in the absence of effects on tumor progression, or even without stimulation of immune responses? In the case of brain tumors, would it be worth repeating a small trial, but attempting to obtain direct evidence of intracranial antitumor immune reactivity, possibly utilizing PET imaging? Which should be the way forward for immunotherapies to cross the bar of generalized clinical implementation?

Sampson et al reported an increased OS of 9 months compared with contemporaneous controls.²⁰ If a comparable difference would be maintained in a larger randomized trial, the use of the EGFRvIII vaccine in suitable patients would be amply justified. The challenge remains how to determine the rational use of EGFRvIII vaccine in the absence of a large randomized, double blind, controlled, phase III trial. Thus, it is important to explore alternatives to study in detail small clinical trials. For example, it would be feasible to perform further small clinical trials that would compare various immunotherapeutic

approaches, and analyze these trials using either adaptive trial designs, or Bayesian designs that simultaneously evaluate efficacy and toxicity.

Clinical statistical challenges remain in how best to assess survival benefits seen in small clinical trials. If we could demonstrate beyond reasonable doubt that those increases are indeed clinically significant it would go a long way to reassure all stakeholders that the benefit is not because of inadvertent bias. In other cases (i.e., at the early stages of treatment for childhood leukemias) it was possible to determine whether, even in the absence of long-term remissions, anticancer agents displayed temporary reductions in the proliferation of neoplastic cells. Such small, yet clinically significant effects led to further improvements, which, during years of continued research led to the ultimate reduction in the morbidity and mortality of diseases such as childhood leukemias. It remains the responsibility of clinicians, oncologists, neurosurgeons, neuroradiologists, as well as basic scientists involved in glioma research, to advance our statistical, oncological, and clinical assessment of small clinical trials, to achieve large therapeutic effect sizes, to benefit the patient community as a whole by being able to extract as much clinically relevant information as possible, and to separate true treatment effects from inadvertent experimental bias and artifacts. Unfortunately for the clinical translational study of glioma therapies, MRI studies have evidenced shortcomings. For example, it is sometimes difficult to determine if MRI images reflect true tumor recurrence or inflammation surrounding the original resection cavity, a phenomenon described as pseudoprogression.³¹ In response to these challenges various groups are now pursuing improved imaging modalities, in some cases accompanied by parallel histologic sampling to improve the accuracy of glioma imaging in the context of translation clinical trials.

In view of evidence in favor of therapeutic activity of anti-EGFRvIII immunotherapy, serious consideration should be given to the combination of this approach with vaccination against other antigens that are also now reporting partial yet significant therapeutic benefits. Just as HIV replication can only be blocked by simultaneously inhibiting three to five central physiological processes needed by the virus to replicate, a similar multipronged approach to the treatment of glioma tumors is predicted to carry stronger potential therapeutic benefits. Indeed, an analysis of current anticancer therapeutics indicates that nonspecific therapeutic agents, such as alkylating agents, antimetabolites, microtubule inhibitors, cytotoxic drugs, and topoisomerase inhibitors, among others continue to be the mainstay of cancer treatment and in many cases particular combinations have been proven to be able to even cure certain cancers.

The remaining clinical challenge is how to develop stable algorithms to determine the level of survival benefit that is indeed clinically significant to reassure all patients, clinicians, and researchers that the benefit is not because of inadvertent noncontrolled experimental biases.

We also need to take into consideration that many nonrandomized immunotherapy trials select for a favorable prognostic group of patients (e.g., only patients with gross total resection, good performance status, etc.) or compare trial data with historical controls. These issues further complicate interpretation of survival time in those studies in comparison with 'standard-of-care' data. Treatment-related immuno-suppression (i.e., lymphopenia) is a

potential additional hurdle regarding the efficacy of immunotherapy. There are now clear data that profound lymphopenia is common, and that it can be prolonged in patients with glioma who have received radiation or chemoradiation. Nevertheless, the finding that specific immune responses can still be induced in subjects treated with standard of care indicates that the clinical consequences of the relationship between lymphopenia and responses to immunization need to be studied further. Clinical trials of any type ought to evaluate these challenges as part of trial design.

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KEY POINTS

- Brain tumor immune therapies are now being tested in large, double-blind, randomized clinical trials.
- The use of glioma-derived antigens, such as EGFRvIII, has provided novel approaches to immunotherapy against brain tumors.
- The engineering of T cells recognizing specific antigens by using chimeric antigen receptors is the latest approach to brain immunotherapy; this approach is not being tested in clinical trials in human patients yet.
- The use of modulators of CTLA-4 and PD1/PDL1 is a further immune modulator that may be of benefit for patients with glioma, and needs to be tested in clinical trials.
- In the future it is likely that the combination of immune therapies acting through complementary mechanisms, i.e., vaccination against whole patients' tumors, vaccination against individual or multiple specific tumor antigens, the use of anti-glioma engineered T cells, together with immune modulatory gene therapy (i.e., Ad-TK/Ad-Flt3L), and powerful blockers of negative regulators of T cell function such as CTLA4-Ig, and antibodies to PD1/PDL1, are likely to achieve the best possible outcomes for patients.

TABLE 1

Vaccine Trials for Glioblastoma Multiforme

Study	Phase	Patients (n)	Number of Injected DCs	DC Loading/Treatment	Trial Result(s)	Ref
Phuphanich et al (2013)	I	17	1×10^7	Peptide epitopes: HER2, TRP-2, gp100, MAGE-I, IL13alpha2 and AIM-2; HLA-A1 and/or HLA-A2	PFS: 16.9 months Median survival: 38.4 months	[32]
Wheeler et al (2008)	II	32	1×10^7 to 4×10^7	Tumor cell lysate	TTP: 208 vs. 167 days for controls	[33]
Yu et al (2001)	I	9	1×10^6	MHC class I peptides	Median survival: 455 vs. 257 days for controls	[34]
Iwami et al (2012)	I	8	1×10^7	IL13R α 2	SD: 1 Regression: 1	[35]
Prins et al (2011)	I	23	1×10^6 to 5×10^6 to 10×10^6	Tumor cell lysate	Median survival: 31.4 months	[36]
Liau et al (2005)	I	12	1×10^6 to 5×10^5 to 1×10^7	DCs loaded with acid-eluted peptides	8 patients re-operated: T-cell infiltration inversely correlated with TGF- β 2 and positively correlated with OS	[37]
Okada et al (2011)	I/II	13	1 or 3×10^7	Peptide epitopes EphA2, IL13alpha2, YKL-40 and gp100	SD: 6, CR: 1, PR: 2 Median survival for recurrent GBM: 12 months	[38]
Sampson et al (2010)	I	12	1×10^8	EGFRvIII-specific peptide conjugated to KLH		[20]
Sampson et al (2009)	I	12	1×10^8	EGFRvIII-specific peptide conjugated to KLH	TTP: 10.2 months Median survival: 18.7 months	[39]
De Vleeschouwer et al (2008)	I/II	56	0.7×10^6 to 1×10^6	Tumor cell lysate	12-month OS: 37.4%, 24-month OS: 14.8%, 36-month OS: 11.1%	[40]
Walker et al (2008)	I	13	1×10^6	Tumor cell lysate	1-year survival: 38%	[41]
Yamanaka et al (2005)*	I/II	24	1×10^7	Tumor cell lysate	PR: 1, MR: 3, SD: 10	[42]
Yamanaka et al (2003)	I/II	10	10×10^6 to 32×10^6	Tumor cell lysate	MR: 2, NC: 4	[43]
Kikuchi et al (2001)	I	8	2.4×10^6 to 8.7×10^6	Tumor cell lysate	SD: 6	[44]
Steiner et al (2004)	Pilot	27	1×10^7	Active immunotherapy GBM cells infected with the Newcastle disease virus	OS: 100 weeks	[45]
Dillman et al (2009)	I/II	36	$1.75 \pm 0.82 \times 10^9$	Adoptive immunotherapy Intratumoral LAKs in GBM after standard therapy	Median survival: 20.5 months	[46]
Dillman et al (2004)	I/II	40	$2.0 \pm 1.0 \times 10^9$ or $2.7 \pm 0.9 \times 10^9$ or $1.8 \pm 0.9 \times 10^9$	Intralesional LAK cell therapy	Median survival: 17.5 months	[47]
Sankhla et al (1996)	I	10	2.0 to 10.0×10^6	LAK and IL-2	PR: 2	[48]
Hayes et al (1995)	I	15	1.2 MIU	LAK and IL-2	Median survival: 53 weeks	[49]
Boiardi et al (1994)	I	9	1.5×10^8	Recombinant IL-2 and LAK	CR: 1, PR: 2, SD: 4	[50]

Study	Phase	Patients (n)	Number of Injected DCs	DC Loading/Treatment	Trial Result(s)	Ref
Jeffes et al (1993)	I	19	2.2×10^9 to 58×10^9	LAK	Median survival: 37 weeks	[51]
Blancher et al (1993)	I	13	18×10^6 or 24×10^6 or 54×10^6	rIL-2 with and without LAK	Tumor progression noted after 4–12 weeks	[52]
Lillehei et al (1991)	I	20	$1.9\text{--}27.5 \times 10^9$	LAK and ASL with rIL-2	Median survival: 63 weeks	[53]
Barba et al (1989)	I	10	$0.9\text{--}21.0 \times 10^9$	IL-2 or LAK	PR: 1	[54]
Jacobs et al (1986)	I	9	1×10^8	IL-2 or LAK	No PR or SD	[55]
Tsuiboi et al (2003)	I	10	$3\text{--}247 \times 10^7$	ATTL cocultured in medium containing interleukin-1, -2, -4, and -6	CR: 1; PR: 4; Median survival: 5 months; Overall response rate: 50%	[56]
Wood et al (2000)	I	9	2×10^7	Lymphocyte stimulation with anti-CD3 and IL-2	PR: 3; Survival >4 years: 2	[57]
Holladay et al (1996)	I	15	8.8×10^6 or 3×10^6	Tumor reactive T cells and IL-2	No PR or SD; Disease-free survival >8 months: 7	[58]
Plantz et al (2000)	I	9	$0.6\text{--}5.5 \times 10^{10}$	Adoptive transfer ex vivo activated T lymphocytes	PR: 3; (1 GBM and 2 grade III survival >4 years)	[59]
Plantz et al (1998)	I	10	17 to 56×10^6	Adoptive transfer ex vivo activated T lymphocytes	SD: 1; 4 patients alive after 1 year	[60]
Sloan et al (2000)	I	19	2×10^7	Adoptive transfer ex vivo activated T lymphocytes	CR: 1; PR: 7; Median survival: 12 months	[61]
Quattrocchi et al (1999)	I	6	1×10^9 TILs + 1×10^5 rIL-2 or 1×10^9 TILs + 4×10^5 rIL-2	Autologous intraleisional TILs and rIL-2 in recurrent MGs	CR: 1, PR: 2	[62]
Tsurushima et al (1999)	I	4	$0.07\text{--}11 \times 10^7$	Cytotoxic T lymphocytes injected via Ommaya tubes	CR: 1; SD: 1	[63]
Kruse et al (1997)	I	5	$1.1\text{--}51.5 \times 10^8$	Alloreactive cytotoxic T lymphocytes and IL-2	Transient toxicities: Survival (AO) >30 months; Survival (AA) >28 months	[64]
Kitahara et al (1987)	I	5	$5.0\text{--}10.4 \times 10^8$	Adoptive immunotherapy IL-2 dependent cytotoxic T lymphocytes	PR: 2; 1 patient alive >104 weeks	[65]
Crane et al (2012)	I	12	N/A	Heat shock protein peptide bound to 96 kD chaperone protein (HSP-96)	Median survival: 47 week in immunologic responders	[66]

* ID or ID and IT

Abbreviations: CR, complete response; DC, dendritic cell; EGFR, EGF receptor; GBM, glioblastoma multiforme; ID, intradermal; IT, intratumoral; LAK, lymphokine activated killers; MR, minor response; NC, no change; OS, overall survival; PFS, progression-free survival; PR, partial response; SD, stable disease; TTP, time to progression

TABLE 2

Ongoing Clinical Trials*

Phase Trial ID	Estimated Completion Date/Enrollment	Sponsor	Protocol
Phase I/II NCT00293423	12/2013; n = 50	University of California, San Francisco	Trial of Heat Shock Protein Peptide Complex-96 (HSPPC-96) vaccine for patients with recurrent high-grade glioma
Phase I NCT01403285	03/2014; n = 25	Immatics Biotechnologies GmbH	Trial of peptide-based glioma vaccine IMA950 in patients with glioblastoma IMA950: multi-peptide vaccine containing 11 tumor-associated peptides (TUMAPs) found in a majority of glioblastomas
Phase I NCT01250470	09/2014; n = 9	Roswell Park Cancer Institute	Study of safety, tolerability and immunological effects of SVN53-67/M57-KLH in patients with Survivin-positive malignant gliomas
Phase I NCT00626015	11/2013; n = 20	John Sampson, Duke University Medical Center	Chemotherapy, radiation therapy, and vaccine (PEP-3-KLH) therapy with basiliximab in treating patients with glioblastoma multiforme that has been removed by surgery
Phase I/II NCT01920191	08/2014; n = 16	University Hospital, Geneva	Study of intradermal IMA950 peptide-based vaccine adjuvanted with intramuscular Poly-ICLC in combination with temozolomide in newly diagnosed HLA-A2 glioblastoma patients
Phase II NCT01814813	04/2016; n = 222	Alliance for Clinical Trials in Oncology	Randomized trial comparing the efficacy of Heat Shock Protein-Peptide Complex-96 (HSPPC-96) vaccine given with bevacizumab versus bevacizumab alone in the treatment of surgically resectable recurrent glioblastoma
			Arm 1, HSPPC-96, concomitant bevacizumab
			Arm 2, HSPPC-96 with bevacizumab at progression
			Active comparator: Arm 3, bevacizumab
Phase II NCT00905060	01/2014; n = 555	University of California, San Francisco	Multi-center, single arm investigation of HSPPC-96 vaccine with temozolomide in patients

Phase Trial ID	Estimated Completion Date/Enrollment	Sponsor	Protocol
			with newly diagnosed glioblastoma
Phase II NCT00643097	06/2016; n = 48	John Sampson, Duke University Medical Center	A complementary trial of an immunotherapy vaccine against tumor-specific EGFRvIII
			Arm I: Patients receive PEP-3-KLH conjugate vaccine and sargramostim (GM-CSF) intradermally on days 1, 15, and 29 and then monthly in the absence of disease progression or unacceptable toxicity
			Arm II: Patients receive placebo vaccine intradermally on days 1, 15, and 29. Patients then receive PEP-3-KLH conjugate vaccine and GM-CSF monthly in the absence of disease progression or unacceptable toxicity
Phase II NCT01498328	06/2015; n = 168	Celldex Therapeutics	Study of rindopepimut/GM-CSF in patients with relapsed EGFRvIII-positive glioblastoma (ReACT)
			Group 1a: bevacizumab naive with bevacizumab + rindopepimut
			Group 1b: bevacizumab naive with bevacizumab + KLH control
			Group 2 and 2C: refractory to bevacizumab
Phase III NCT01498328	11/2016; n = 440	Celldex Therapeutics	International, randomized, double-blind, controlled study of rindopepimut/GM-CSF with adjuvant temozolomide in newly diagnosed, surgically resected, EGFRvIII-positive glioblastoma (ACT IV)
			Comparator: KLH plus temozolomide
			2 id injections 2 wk apart, followed by monthly injections until tumor progression or intolerance
Phase II NCT00458601	12/2013; n = 82	Celldex Therapeutics	CDX-110 in patients with glioblastoma multiforme (ACT III)
Phase I NCT01058850	9/2013; n = 3	Paul Graham Fisher, Stanford University	Rindopepimut after conventional radiation in children with diffuse intrinsic pontine gliomas

Phase Trial ID	Estimated Completion Date/Enrollment	Sponsor	Protocol
Phase II NCT00323115	4/2008; n = 11	Dartmouth-Hitchcock Medical Center	Phase II feasibility study of DC vaccination for newly diagnosed GBM
Phase I NCT00612001	1/2011; n = 8	UCLA; Jonsson Compr. Cancer Center	Vaccine therapy for patients with malignant glioma
Phase I NCT00639639	6/2016; n = 16	J. Sampson, Duke Univ. Medical Center	Vaccine therapy in treating patients with newly diagnosed glioblastoma multiforme
Phase II NCT01213407	3/2011; n = 56	Trimed Biotech GmbH	DC cancer vaccine for high-grade glioma (GBM-Vax)
Phase I/II NCT00846456	12/2013; n = 20	Oslo University Hospital	Safe study of dendritic cell (DC) based therapy targeting tumor stem cells in glioblastoma
Phase II NCT01006044	10/2013; n = 26	Clinica Universidad de Navarra, Univ. de Navarra	Efficacy & safety of autologous dendritic cell vaccination in glioblastoma multiforme after complete surgical resection
Phase I NCT00068510	9/2013; n = 28	UCLA; Jonsson Compr. Cancer Center	Vaccine therapy in treating patients with malignant glioma
Phase I/II NCT01291420	2/2014; n = 10	University Hospital, Antwerp	DC vaccination for patients with solid tumors
Phase III NCT00045968	9/2014; n = 300	Northwest Biotherapeutics	Study of a drug [DCVax-L] to treat newly diagnosed GBM brain cancer
Phase II NCT01280552	10/2014; n = 200	ImmunoCellular Therapeutics, Ltd.	A study of ICT-107 immunotherapy in glioblastoma multiforme (GBM)
Phase I NCT00890032	9/2015; n = 50	J. Sampson, Duke Univ. Medical Center	Vaccine therapy in treating patients undergoing surgery for recurrent glioblastoma multiforme
Phase II NCT00905060	1/2014; n = 55	UCSF	HSPPC-96 vaccine with temozolomide in patients with newly diagnosed GBM (HeatShock)
Phase I/II NCT00293423	12/2013; n = 50	UCSF	GP96 heat shock protein-peptide complex vaccine in treating patients with recurrent or progressive glioma

Abbreviations: DC, dendritic cells; GBM, glioblastoma multiforme

* Information from www.clinicaltrials.gov