Title

Vaccine therapies in malignant glioma.

Journal

Current neurology and neuroscience reports, 15(1)

ISSN

1528-4042

Authors

Oh, Taemin
Sayegh, Eli T
Fakurnejad, Shayan
et al.

Publication Date

2015

DOI

10.1007/s11910-014-0508-y

Peer reviewed

HHS Public Access

Author manuscript

Curr Neurol Neurosci Rep. Author manuscript; available in PMC 2016 January 01.

Published in final edited form as:

Curr Neurol Neurosci Rep. 2015 January ; 15(1): 508. doi:10.1007/s11910-014-0508-y.

Vaccine Therapies in Malignant Glioma

Taemin Oh, BA[1], **Eli T. Sayegh, BS**[1], **Shayan Fakurnejad, BS**[1], **Daniel Oyon, BS, MS**[1], **Jonathan Balquiedra Lamano, BS**[1], **Joseph David DiDomenico, BS**[1], **Orin Bloch, MD**[1], and **Andrew T. Parsa, MD, PhD**[1]

[1]Department of Neurological Surgery, Northwestern University Feinberg School of Medicine, 676 N. St. Clair Street, Suite 2210, Chicago, IL 60611

Abstract

Glioblastoma is a grade IV astrocytoma that is widely accepted in clinical neurosurgery as being an extremely lethal diagnosis. Long-term survival rates remain dismal and, even when tumors undergo gross resection with confirmation of total removal on neuroimaging, they invariably recur with even greater virulence. Standard therapeutic modalities as well as more contemporary treatments have largely resulted in disappointing improvements. However, the therapeutic potential of vaccine immunotherapy for malignant glioma should not be underestimated. In contrast to many of the available treatments, vaccine immunotherapy is unique because it offers the means of delivering treatment that is highly specific to both the patient and the tumor. Peptide, heat-shock proteins, and dendritic cell vaccines collectively encapsulate the majority of research efforts involving vaccine-based treatment modalities. In this review, important recent findings for these vaccine types are discussed in the context of ongoing clinical trials. Broad challenges to immunotherapy are also considered.

Keywords

glioma; glioblastoma; immunotherapy; vaccine

Introduction

Glioblastoma (GBM) is a high-grade glial tumor synonymous with significant morbidity and mortality, and an exceptionally poor clinical course. Contemporary management strategies - which involve maximum gross total resection combined with adjuvant Temozolomide chemo-radiotherapy - yield a bleak median overall survival (OS) of just 14.6 months [1]. By and large, attempts at developing a more sustainable treatment solution have been met with limited success, despite decades of intense research efforts [2]. The source of this discrepancy is manifold including, but not limited to, intra-tumoral heterogeneity [3], brain tumor stem cells [4], and genomic subtypes of varying virulence [5]. In the contemporary era, GBM thus remains a conundrum for which optimum treatment has yet to be discovered.

Corresponding Author: Andrew T. Parsa, M.D. Ph.D, Department of Neurological Surgery, Northwestern University, Feinberg School of Medicine, 676 N. St. Clair Street, Suite 2210, Chicago, IL 60611-2911, Phone: (312) 695-1801, Fax: (312) 695-0225, aparsa@nmff.org.

The elegant complexity of GBM tumor biology and immunology indicate that a “magic bullet” solution does not exist. The more probable scenario is that successful management of this tumor will require a multi-factorial approach designed to challenge tumor growth on multiple fronts. In that fashion, vaccine immunotherapy for GBM has often emerged as a promising addition to the wide spectrum of available treatment strategies. When compared to other therapeutic modalities, vaccine immunotherapy is particularly compelling because it can induce a highly patient-specific, anti-tumor response while carrying minimal toxicity [6, 7]. Furthermore, it promises to induce a paradigmatic shift in which GBM, widely recognized as one of the most lethal human tumors, instead becomes known as a chronic disease state that can be managed with medication. Much like in the management of diabetes or hypertension, patients would follow up with their physicians for scheduled check-ups, undergo monitoring of their therapeutic progress, and alter their therapeutic regimen accordingly. Personalized to the patient and individualized to the tumor, vaccine immunotherapy thus integrates well with the modern concept of “personalized” medicine, as it offers the means to approach each GBM as a unique entity requiring unique interventions.

While the translational application of a vaccine to treat brain tumors is a modern concept, the fundamental principles behind tumor vaccines largely adhere to those discovered by Edward Jenner in 1796. Analogous to the vaccines utilized in the realm of infectious disease, tumor vaccines are designed to present tumor-associated antigens (TAAs) to the host and to stimulate a pro-inflammatory anti-tumor response. Once the vaccine is injected, TAAs are initially ingested by antigen presenting cells (APCs), which subsequently present these peptides on Major Histocompatibility Complexes I (MHC I) and II (MHC II). Naïve T cells bind accordingly, with $CD8^+$ T cells and $CD4^+$ T cells attaching to MHC I and MHC II, respectively. Naïve $CD8^+$ T cells then undergo differentiation into cytotoxic T lymphocytes (CTLs) that can lyse pathogens and tumor cells, thus establishing these cells as the “effector” arm of the immune response. Naïve $CD4^+$ T cells, on the other hand, adopt either a Th_1 or Th_2 phenotype depending on the release of specific cytokines and lymphokines (e.g. interleukins). While both Th phenotypes are respectively crucial to physiologic immune function, it is important to note that Th_1 favors the effector $CD8^+$ response while the Th_2 phenotype engenders a humoral, antibody-mediated immune response [8].

In that manner, tumor vaccines instigate an immune response that is both tumor-specific and patient-specific [9]. Currently, vaccine therapies for GBM have been successfully investigated in clinical trials, with promising results so far highlighting their potential for future therapeutic integration. Tumor vaccines are categorized according to the manner in which they are delivered. Peptide, dendritic cell (DC), and heat shock protein (HSP) vaccines represent the most well-known vaccines. In the following sections, this article will review recent key findings and discuss ongoing clinical trials for these vaccine types.

Peptide Vaccines

The eponymously named peptide vaccines constitute one of the major vaccine types in GBM immunotherapy, and involve the direct administration of TAAs. In theory, introducing TAAs into the host effectively primes the systemic immune response to target all body

tissues expressing that antigen. In order to maximize specificity against tumor, it is therefore critical to select only those TAAs that are highly expressed in GBM but not in normal tissue. Although TAAs can be extracted from raw tumor tissue, synthetic derivations of known tumor epitopes have found greater popularity [10]. Unlike DC or HSP vaccines, peptide vaccines are not inherently optimized to generate an immune environment conducive to antigenic targeting and eradication of tumor tissue. As a result, these vaccines are often employed in conjunction with immuno-stimulatory adjuvants such as Toll-Like Receptor (TLR) agonists in an attempt to enhance the immune response [10–12].

Within the glioma literature, aberrant expression of TAAs such as gp100, TRP-2, AIM-2, MAGE-1, and others have been described [13]. However, peptide vaccines directed against the Epidermal Growth Factor Receptor variant III (EGFRvIII) are perhaps the most widely recognized. The association between EGFR mutations and cancer has a long history, having been implicated not only in the oncogenesis of lung and colon cancer but also in gliomagenesis [14–17]. In GBM, EGFRvIII is a constitutively active protein that is not expressed in normal human brain [18]. So far, published results for clinical trials using EGFRvIII peptide vaccines have involved administration of Rindopepimut (CDX-110) [11, 19]. Success initially found in experimental animal studies using a peptide vaccine targeting the mutated EGFRvIII domain (known as PEP-3-KLH) facilitated entry of this vaccine into clinical trials [20]. In 2010, Sampson *et al.* completed a multi-center Phase II trial in which 18 eligible patients with EGFRvIII-expressing GBM received intradermal injections of CDX-110. Median OS was 26.0 months while median progression-free survival (PFS) was 14.2 months. When compared to a matched control cohort, patients receiving vaccination demonstrated significantly longer OS than those who had not ($p=0.001$). Several Phase I–III trials are ongoing to further validate the therapeutic potential of Rindopepimut, albeit in combination with GM-CSF (NCT01498328, NCT01480479) [11, 12].

However, one of the major pitfalls of peptide vaccines is their limited external generalizability. For instance, the vast majority of peptide vaccines are restricted to the HLA-A*02 haplotype, which calls into question their utility in GBM patients who present with different haplotypes [21, 13]. Additionally, peptide vaccines lead to an imperfect solution, as tumor recurrence post-vaccination often requires alteration of the therapeutic approach. Sampson and colleagues demonstrated that when tumors recurred, 82% of patients demonstrated loss of EGFRvIII expression. While this finding suggests that the vaccine successfully targeted $EGFRvIII^{+}$ tumor cells, it also implies that vaccine treatment led to selective propagation of $EGFRvIII^{-}$ cells [18]. Future directions with peptide vaccinations may thus require targeting of multiple epitopes in order to counteract the inherent heterogeneity of GBM tumor cells. In that manner, contemporary vaccines such as IMA950 (NCT01403285, NCT22418738, NCT01222221, NCT01920191), which consists of a collection of 11 synthetically derived peptides, may represent a step in the right direction [11, 19].

Heat Shock Protein (HSP) Vaccine

HSPs are primarily involved in the regulation of protein chaperoning and protein folding [22]. However, much less recognized is the fact that HSPs also play a role in the immune

response [23, 24]. HSP vaccines, which represent a particular type of peptide vaccine, are designed to exploit this biologic relationship. In principle, APCs treat HSPs as if they are any other antigen: APCs internalize the HSPs through receptor-mediated endocytosis (e.g. CD14, CD91) and subsequently present the peptides on MHC complexes to generate immunogenicity against those antigens [25–28, 23, 29–31]. As such, HSP vaccines are designed as TAAs conjugated to HSPs, with the former designed to incite a specific anti-tumor response and the latter designed to enhance the inflammatory response.

The majority of contemporary clinical trials for HSP vaccines revolve around TAAs bound to a 96 kD chaperone heat shock protein, otherwise known as HSP protein complex-96 vaccine (HSPPC-96; Prophage) [12]. In 2012, Crane *et al.* reported Phase I results demonstrating that administration of this vaccine induced a significant immune response, as evidenced by the fact that tumor biopsies revealed marked microenvironment infiltration with $CD4^+$, $CD8^+$, and $CD56^+$ T cells. Furthermore, of the 12 patients who were treated, 11 responded well to the drug, demonstrating a median OS of 47 weeks [32]. In 2014, the same group published Phase II results, in which 41 patients were treated with the HSPPC-96 vaccine. Median OS, at 42.6 weeks, was comparable to the Phase I results, which represents an improvement over the benchmark of 14.6 months [33]. Of interest, Wu *et al.* recently described expression of HSP47 as a novel TAA. More specifically, they found that HSP47 expression was significantly increased in GBM tissue but not in normal tissue, and that patients who were able to propagate a CTL response against HSP47 had significantly prolonged PFS and OS [34]. As such, future research with other HSP antigens such as HSP47 may provide alternative targets for HSP-based vaccine therapies.

Dendritic Cell Vaccine

Dendritic cells (DC) comprise a subset of immune cells that serve as “professional” APCs, and these cells play a substantial role in generating both the CD4 and CD8 immune responses. Particularly germane to vaccine immunotherapy, DC vaccines are known for their robust ability to immunologically present glioma antigens, activate cytotoxic $CD8^+$ cells, and induce tumor cell death [35, 36]. Conceptually, the vast majority of DC-based vaccines require extraction of autologous DCs from the patient, DC “loading” or “pulsing” with tumor lysates or peptides, and subsequent re-introduction into the patient.

DC vaccines carry a long investigational track record within the history of GBM immunotherapy and, as a result, perhaps represent one of the most familiar vaccine modalities [37–42]. In a Phase I clinical trial published in 2011, 23 GBM patients received biweekly treatment with pulsed DCs followed by adjuvant treatment with either imiquimod or poly-ICLC. Median OS was 31.4 months and rates of 3-year survival were 47%. Interestingly, the authors noted that patients possessing tumors with mesenchymal gene expression patterns appeared to be particularly susceptible to this treatment approach [43]. This perhaps serves as an indication that particular vaccine types may find therapeutic superiority based on the tumor’s genetic composition. In another Phase I trial published in 2013, 21 patients were enrolled to receive DCs pulsed with an assortment of TAAs. Treatment resulted in a median OS and PFS of 38.4 and 16.9 months, respectively [13].

Currently, several Phase II (NCT01280552, NCT01635283, NCT01204684) and Phase III trials (NCT00045968) for DC vaccines are ongoing.

More recently, the literature has placed greater emphasis on fine-tuning the DC vaccination protocol. In 2013, Prins *et al.* compared the efficacy and safety of two independent protocols for DC vaccine delivery in a Phase I trial. One cohort of 28 patients was treated with DCs that had been pulsed with autologous tumor lysates, while the other cohort of 6 patients was treated with DCs that had been pulsed with synthetic glioma antigen peptides. The study found that while both treatment arms resulted in minimal toxicity and an adequate antitumor response, administration of DCs pulsed with autologous tumor lysates was associated with a stronger immune response against tumor [44]. Other studies have also focused on identifying potentially critical biomarkers to measure immune response against tumor, including phospho-STAT [45], regulatory T cells [44], and cytotoxic T-lymphocyte-associated protein 4 [46]. Characterization of such markers will represent a critical step in the right direction, as inherent GBM-induced immunosuppression represents one of the most significant barriers to treatment efficacy in immunotherapy.

Challenges to Vaccine Immunotherapy

As is often the case with any investigational therapeutic regimen, vaccine immunotherapy is not without its unique set of challenges. First and foremost, the extreme heterogeneity of GBM raises significant concerns. Current clinical data for the use of monovalent vaccines indicates inadequate tumor control [9, 47]. Monovalent vaccines additionally suffer from the potential for tumors to select for those that are resistant to the vaccine, as illustrated previously in the aforementioned EGFRvIII trial [18, 12]. This is perhaps unsurprising: GBM tumors demonstrate a natural tendency towards harboring multiple different tumor cell populations, each with their own unique set of mutations, and it is highly unlikely that targeting a single antigen will result in successful tumor control. Beyond the heterogeneity of individual tumors, recent molecular classification of GBM into the proneural, neural, classical, and mesenchymal subtypes [5] has introduced even greater complexity to the disease, and it remains unclear how each subtype responds to the various immunotherapies mentioned above. In that manner, future therapeutic investigations must operate from the basic premise that a “one-size-fits-all” treatment does not exist. Polyvalent immunizations and other multi-faceted therapeutic approaches that target a range of tumor cell populations will thus be important to consider.

Furthermore, the presence of MHC I^- glioma tumor cells, which make up 30–60% of the entire tumor population [48], hinders the effectiveness of immunotherapies since MHC I molecules on the tumor cell surface are essential for CTL-mediated localization and eradication of cancer cells. To overcome this, utilization of adjuvants that facilitate targeting of these otherwise immune-evasive tumor cell types has been suggested as a potential strategy [48]. The combined use of glioma vaccines with chemotherapy or other biological therapies that can recruit NK or macrophages may act to supplement the immune response, and eradicate MHC I^- glioma tumor cells as well [48]. Additionally, adaptive immunotherapy, which relies on *ex vivo* activation and expansion of autologous immune cells that are later infused into the patient, is another potential adjuvant that could act to

overcome this challenge. It has been shown that autologous lymphocytes can be directed to generate NK cells in the presence of IL-2, which can then be intratumorally injected to combat the MHC I^- glioma tumor cells [12].

Secondly, it is also important to note that while vaccine therapies have demonstrated efficacy in immune activation, with associated improvements in clinical outcome in GBM patients, improving our understanding of the interplay between immunotherapy and current cancer management protocols is crucial. Chemoradiation and immunotherapy must be combined in a strategic fashion so as to maintain, or even augment, the efficacy of both interventions. Myelosuppression and lymphodepletion, which are a common result of the natural GBM disease process as well as being adverse effects of chemotherapy and radiation, generally present large obstacles for immunotherapy [12]. In order to minimize this two-pronged attack on the immune response, local administration of chemotherapy may be preferable to systemic administration, especially for intracranial tumors [12]. However, it has also been postulated that exploitation of postoperative inflammation and enhanced tumor-infiltrating immune cell fractions may be possible, indicating that timing will be crucial when combining immunotherapy with current standard-of-care [12].

Enhanced efficiency may also be gained by way of adjuvants targeting immunosuppressive mediators that induce GBM immune escape. For example, interleukins (IL) are a family of lymphokines that influence the differentiation of naïve T cells. Of particular interest, IL-4, IL-10, and IL-13 lead to a bias towards Th_2 differentiation, the same phenotype generally induced by GBM [49, 50]. Additionally, TGF-β is known to inhibit both the innate and adaptive immune response, and elevated levels of TGF-β are commonly associated with glioma [51]. Lastly, programmed death ligand-1 (PD-L1) has been proven to be a key immunosuppression factor that is highly expressed in glioma [52, 53]. PD-L1, expressed on both glioma and lymphocytes exposed to glioma, binds to its respective receptor, programmed cell death protein 1 (PD-1), on T lymphocytes, and results in T-cell apoptosis [52].

Of interest, the PI3K pathway has been shown to mediate the production of these immunological substances [53–55]. As such, PI3K inhibitors have recently been investigated in early Phase clinical trials for the treatment of glioma [56, 57]. However, utilization of PI3K pathway inhibitors as adjuvants to immunotherapy must be approached with some caution. Interferons, which are pro-inflammatory proteins that can inhibit tumor growth, are decreased in response to PI3K pathway inhibitors [58–60]. PI3K pathway inhibitors also increase the proportion of regulatory T cells (T_{regs}) by specifically targeting and inhibiting proliferation of other T cell subsets [61]. This further diminishes the release of pro-inflammatory cytokines and shifts the immune phenotype towards Th_2 [62]. As such, while adjuvants such as PI3K pathway inhibitors are potentially promising additions to enhance vaccine immunotherapy, a fine balance must be struck between their pro-inflammatory and anti-inflammatory responses.

Conclusion

Vaccine immunotherapy for malignant glioma offers the means of delivering treatment that is highly specific to both the patient and the tumor. Mainly comprised of peptide, heat-shock proteins, and dendritic cell vaccines, this emerging therapeutic arm has proven to be a safe and effective way to combat some of the challenges facing standard-of-care for GBM patients. As new potential targets are uncovered and existing trials continue on to Phases II and III, the clinical benefit and role of immunotherapy in the management of malignant glioma will become clearer. Encouraging data from early phase trials, as well as the challenges they have presented, should endorse further research into this treatment modality.

VII. References

**1. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. The New England journal of medicine. 2005; 352(10):987–96. Establishes the current standard-of-care for treatment of glioblastoma. 10.1056/NEJMoa043330 [PubMed: 15758009]

2. Selznick LA, Shamji MF, Fecci P, Gromeier M, Friedman AH, Sampson J. Molecular strategies for the treatment of malignant glioma--genes, viruses, and vaccines. Neurosurgical review. 2008; 31(2): 141–55. discussion 55. 10.1007/s10143-008-0121-0 [PubMed: 18259789]

3. Vartanian A, Singh SK, Agnihotri S, Jalali S, Burrell K, Aldape KD, et al. GBM's multifaceted landscape: highlighting regional and microenvironmental heterogeneity. Neuro-oncology. 2014; 16(9):1167–75.10.1093/neuonc/nou035 [PubMed: 24642524]

4. Schonberg DL, Lubelski D, Miller TE, Rich JN. Brain tumor stem cells: Molecular characteristics and their impact on therapy. Molecular aspects of medicine. 201310.1016/j.mam.2013.06.004

5. Verhaak RG, Hoadley KA, Purdom E, Wang V, Qi Y, Wilkerson MD, et al. Integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR, and NF1. Cancer cell. 2010; 17(1):98–110.10.1016/j.ccr.2009.12.020 [PubMed: 20129251]

6. Aguilar LK, Arvizu M, Aguilar-Cordova E, Chiocca EA. The spectrum of vaccine therapies for patients with glioblastoma multiforme. Current treatment options in oncology. 2012; 13(4):437–50.10.1007/s11864-012-0208-2 [PubMed: 22903697]

7. Tanaka S, Louis DN, Curry WT, Batchelor TT, Dietrich J. Diagnostic and therapeutic avenues for glioblastoma: no longer a dead end? Nature reviews Clinical oncology. 2012; 10(1):14–26.10.1038/nrclinonc.2012.204

8. Liao W, Lin JX, Leonard WJ. IL-2 family cytokines: new insights into the complex roles of IL-2 as a broad regulator of T helper cell differentiation. Current opinion in immunology. 2011; 23(5):598–604.10.1016/j.coi.2011.08.003 [PubMed: 21889323]

9. Tanaka S, Louis DN, Curry WT, Batchelor TT, Dietrich J. Diagnostic and therapeutic avenues for glioblastoma: no longer a dead end? Nature reviews Clinical oncology. 2013; 10(1):14–26.10.1038/nrclinonc.2012.204

10. Mohme M, Neidert MC, Regli L, Weller M, Martin R. Immunological challenges for peptide-based immunotherapy in glioblastoma. Cancer treatment reviews. 2014; 40(2):248–58.10.1016/j.ctrv.2013.08.008 [PubMed: 24064197]

11. Sayegh ET, Oh T, Fakurnejad S, Bloch O, Parsa AT. Vaccine therapies for patients with glioblastoma. Journal of neuro-oncology. 201410.1007/s11060-014-1502-6

12. Jackson C, Ruzevick J, Brem H, Lim M. Vaccine strategies for glioblastoma: progress and future directions. Immunotherapy. 2013; 5(2):155–67.10.2217/imt.12.155 [PubMed: 23413907]

13. Phuphanich S, Wheeler CJ, Rudnick JD, Mazer M, Wang H, Nuno MA, et al. Phase I trial of a multi-epitope-pulsed dendritic cell vaccine for patients with newly diagnosed glioblastoma. Cancer immunology, immunotherapy : CII. 2013; 62(1):125–35.10.1007/s00262-012-1319-0 [PubMed: 22847020]

14. Wong AJ, Ruppert JM, Bigner SH, Grzeschik CH, Humphrey PA, Bigner DS, et al. Structural alterations of the epidermal growth factor receptor gene in human gliomas. Proceedings of the National Academy of Sciences of the United States of America. 1992; 89(7):2965–9. [PubMed: 1557402]

15. Humphrey PA, Wong AJ, Vogelstein B, Friedman HS, Werner MH, Bigner DD, et al. Amplification and expression of the epidermal growth factor receptor gene in human glioma xenografts. Cancer research. 1988; 48(8):2231–8. [PubMed: 3258189]

16. Humphrey PA, Wong AJ, Vogelstein B, Zalutsky MR, Fuller GN, Archer GE, et al. Anti-synthetic peptide antibody reacting at the fusion junction of deletion-mutant epidermal growth factor receptors in human glioblastoma. Proceedings of the National Academy of Sciences of the United States of America. 1990; 87(11):4207–11. [PubMed: 1693434]

17. Kuan CT, Wikstrand CJ, Bigner DD. EGF mutant receptor vIII as a molecular target in cancer therapy. Endocrine-related cancer. 2001; 8(2):83–96. [PubMed: 11397666]

**18. Sampson JH, Heimberger AB, Archer GE, Aldape KD, Friedman AH, Friedman HS, et al. Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2010; 28(31): 4722–9. Provides evidence supporting immunological escape following immunotherapy, highlighting a demanding challenge to current immunotherapeutic approaches. 10.1200/JCO. 2010.28.6963 [PubMed: 20921459]

19. Del Vecchio CA, Li G, Wong AJ. Targeting EGF receptor variant III: tumor-specific peptide vaccination for malignant gliomas. Expert review of vaccines. 2012; 11(2):133–44.10.1586/erv. 11.177 [PubMed: 22309662]

20. Heimberger AB, Crotty LE, Archer GE, Hess KR, Wikstrand CJ, Friedman AH, et al. Epidermal growth factor receptor VIII peptide vaccination is efficacious against established intracerebral tumors. Clinical cancer research : an official journal of the American Association for Cancer Research. 2003; 9(11):4247–54. [PubMed: 14519652]

21. Neidert MC, Schoor O, Trautwein C, Trautwein N, Christ L, Melms A, et al. Natural HLA class I ligands from glioblastoma: extending the options for immunotherapy. Journal of neuro-oncology. 2013; 111(3):285–94.10.1007/s11060-012-1028-8 [PubMed: 23263746]

22. Agashe VR, Hartl FU. Roles of molecular chaperones in cytoplasmic protein folding. Seminars in cell & developmental biology. 2000; 11(1):15–25.10.1006/scdb.1999.0347 [PubMed: 10736260]

23. Nishikawa M, Takemoto S, Takakura Y. Heat shock protein derivatives for delivery of antigens to antigen presenting cells. International journal of pharmaceutics. 2008; 354(1–2):23–7.10.1016/ j.ijpharm.2007.09.030 [PubMed: 17980980]

24. Basu S, Binder RJ, Suto R, Anderson KM, Srivastava PK. Necrotic but not apoptotic cell death releases heat shock proteins, which deliver a partial maturation signal to dendritic cells and activate the NF-kappa B pathway. International immunology. 2000; 12(11):1539–46. [PubMed: 11058573]

25. Pawaria S, Binder RJ. CD91-dependent programming of T-helper cell responses following heat shock protein immunization. Nature communications. 2011; 2:521.10.1038/ncomms1524

26. Asea A, Rehli M, Kabingu E, Boch JA, Bare O, Auron PE, et al. Novel signal transduction pathway utilized by extracellular HSP70: role of toll-like receptor (TLR) 2 and TLR4. The Journal of biological chemistry. 2002; 277(17):15028–34.10.1074/jbc.M200497200 [PubMed: 11836257]

27. Basu S, Binder RJ, Ramalingam T, Srivastava PK. CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70, and calreticulin. Immunity. 2001; 14(3):303–13. [PubMed: 11290339]

28. Asea A, Kraeft SK, Kurt-Jones EA, Stevenson MA, Chen LB, Finberg RW, et al. HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. Nature medicine. 2000; 6(4):435–42.10.1038/74697

29. Singh-Jasuja H, Toes RE, Spee P, Munz C, Hilf N, Schoenberger SP, et al. Cross-presentation of glycoprotein 96-associated antigens on major histocompatibility complex class I molecules requires receptor-mediated endocytosis. The Journal of experimental medicine. 2000; 191(11): 1965–74. [PubMed: 10839811]

30. Suto R, Srivastava PK. A mechanism for the specific immunogenicity of heat shock protein-chaperoned peptides. Science. 1995; 269(5230):1585–8. [PubMed: 7545313]

31. See AP, Pradilla G, Yang I, Han S, Parsa AT, Lim M. Heat shock protein-peptide complex in the treatment of glioblastoma. Expert review of vaccines. 2011; 10(6):721–31.10.1586/erv.11.49 [PubMed: 21692695]

32. Crane CA, Han SJ, Ahn B, Oehlke J, Kivett V, Fedoroff A, et al. Individual patient-specific immunity against high-grade glioma after vaccination with autologous tumor derived peptides bound to the 96 KD chaperone protein. Clinical cancer research : an official journal of the American Association for Cancer Research. 2013; 19(1):205–14.10.1158/1078-0432.CCR-11-3358 [PubMed: 22872572]

33. Bloch O, Crane CA, Fuks Y, Kaur R, Aghi MK, Berger MS, et al. Heat-shock protein peptide complex-96 vaccination for recurrent glioblastoma: a phase II, single-arm trial. Neuro-oncology. 2014; 16(2):274–9.10.1093/neuonc/not203 [PubMed: 24335700]

34. Wu ZB, Cai L, Qiu C, Zhang AL, Lin SJ, Yao Y, et al. CTL responses to HSP47 associated with the prolonged survival of patients with glioblastomas. Neurology. 2014; 82(14):1261–5.10.1212/WNL.0000000000000290 [PubMed: 24623841]

35. Cohn L, Delamarre L. Dendritic cell-targeted vaccines. Frontiers in immunology. 2014; 5:255.10.3389/fimmu.2014.00255 [PubMed: 24910635]

36. Anguille S, Smits EL, Lion E, van Tendeloo VF, Berneman ZN. Clinical use of dendritic cells for cancer therapy. The Lancet Oncology. 2014; 15(7):e257–67.10.1016/S1470-2045(13)70585-0 [PubMed: 24872109]

37. De Vleeschouwer S, Fieuws S, Rutkowski S, Van Calenbergh F, Van Loon J, Goffin J, et al. Postoperative adjuvant dendritic cell-based immunotherapy in patients with relapsed glioblastoma multiforme. Clinical cancer research : an official journal of the American Association for Cancer Research. 2008; 14(10):3098–104.10.1158/1078-0432.CCR-07-4875 [PubMed: 18483377]

38. Kikuchi T, Akasaki Y, Abe T, Fukuda T, Saotome H, Ryan JL, et al. Vaccination of glioma patients with fusions of dendritic and glioma cells and recombinant human interleukin 12. J Immunother. 2004; 27(6):452–9. [PubMed: 15534489]

39. Liau LM, Black KL, Martin NA, Sykes SN, Bronstein JM, Jouben-Steele L, et al. Treatment of a patient by vaccination with autologous dendritic cells pulsed with allogeneic major histocompatibility complex class I-matched tumor peptides. Case Report. Neurosurgical focus. 2000; 9(6):e8. [PubMed: 16817691]

40. Liau LM, Prins RM, Kiertscher SM, Odesa SK, Kremen TJ, Giovannone AJ, et al. Dendritic cell vaccination in glioblastoma patients induces systemic and intracranial T-cell responses modulated by the local central nervous system tumor microenvironment. Clinical cancer research : an official journal of the American Association for Cancer Research. 2005; 11(15):5515–25.10.1158/1078-0432.CCR-05-0464 [PubMed: 16061868]

41. Okada H, Kalinski P, Ueda R, Hoji A, Kohanbash G, Donegan TE, et al. Induction of CD8+ T-cell responses against novel glioma-associated antigen peptides and clinical activity by vaccinations with {alpha}-type 1 polarized dendritic cells and polyinosinic-polycytidylic acid stabilized by lysine and carboxymethylcellulose in patients with recurrent malignant glioma. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2011; 29(3):330–6.10.1200/JCO.2010.30.7744 [PubMed: 21149657]

42. Sampson JH, Archer GE, Mitchell DA, Heimberger AB, Herndon JE 2nd, Lally-Goss D, et al. An epidermal growth factor receptor variant III-targeted vaccine is safe and immunogenic in patients with glioblastoma multiforme. Molecular cancer therapeutics. 2009; 8(10):2773–9.10.1158/1535-7163.MCT-09-0124 [PubMed: 19825799]

43. Prins RM, Soto H, Konkankit V, Odesa SK, Eskin A, Yong WH, et al. Gene expression profile correlates with T-cell infiltration and relative survival in glioblastoma patients vaccinated with dendritic cell immunotherapy. Clinical cancer research : an official journal of the American Association for Cancer Research. 2011; 17(6):1603–15.10.1158/1078-0432.CCR-10-2563 [PubMed: 21135147]

44. Prins RM, Wang X, Soto H, Young E, Lisiero DN, Fong B, et al. Comparison of glioma-associated antigen peptide-loaded versus autologous tumor lysate-loaded dendritic cell vaccination in

malignant glioma patients. J Immunother. 2013; 36(2):152–7.10.1097/CJI.0b013e3182811ae4 [PubMed: 23377664]

45. Everson RG, Jin RM, Wang X, Safaee M, Scharnweber R, Lisiero DN, et al. Cytokine responsiveness of CD8(+) T cells is a reproducible biomarker for the clinical efficacy of dendritic cell vaccination in glioblastoma patients. Journal for immunotherapy of cancer. 2014; 2:10.10.1186/2051-1426-2-10 [PubMed: 24883189]
46. Fong B, Jin R, Wang X, Safaee M, Lisiero DN, Yang I, et al. Monitoring of regulatory T cell frequencies and expression of CTLA-4 on T cells, before and after DC vaccination, can predict survival in GBM patients. PloS one. 2012; 7(4):e32614.10.1371/journal.pone.0032614 [PubMed: 22485134]
47. Jackson C, Ruzevick J, Phallen J, Belcaid Z, Lim M. Challenges in immunotherapy presented by the glioblastoma multiforme microenvironment. Clinical & developmental immunology. 2011; 2011:732413.10.1155/2011/732413 [PubMed: 22190972]
48. Yamanaka R. Cell- and peptide-based immunotherapeutic approaches for glioma. Trends in molecular medicine. 2008; 14(5):228–35.10.1016/j.molmed.2008.03.003 [PubMed: 18403264]
49. Zisakis A, Piperi C, Themistocleous MS, Korkolopoulou P, Boviatsis EI, Sakas DE, et al. Comparative analysis of peripheral and localised cytokine secretion in glioblastoma patients. Cytokine. 2007; 39(2):99–105.10.1016/j.cyto.2007.05.012 [PubMed: 17697783]
50. Kumar R, Kamdar D, Madden L, Hills C, Crooks D, O'Brien D, et al. Th1/Th2 cytokine imbalance in meningioma, anaplastic astrocytoma and glioblastoma multiforme patients. Oncology reports. 2006; 15(6):1513–6. [PubMed: 16685388]
51. Flavell RA, Sanjabi S, Wrzesinski SH, Licona-Limon P. The polarization of immune cells in the tumour environment by TGFbeta. Nature reviews Immunology. 2010; 10(8):554–67.10.1038/nri2808
52. Dong H, Strome SE, Salomao DR, Tamura H, Hirano F, Flies DB, et al. Tumor-associated B7-H1 promotes T-cell apoptosis: a potential mechanism of immune evasion. Nature medicine. 2002; 8(8):793–800.10.1038/nm730
**53. Parsa AT, Waldron JS, Panner A, Crane CA, Parney IF, Barry JJ, et al. Loss of tumor suppressor PTEN function increases B7-H1 expression and immunoresistance in glioma. Nature medicine. 2007; 13(1):84–8. Provides insight into the challenges to immunotherapies, and thus potential improvements for current modalities. 10.1038/nm1517
54. Grehan JF, Levay-Young BK, Fogelson JL, Francois-Bongarcon V, Benson BA, Dalmasso AP. IL-4 and IL-13 induce protection of porcine endothelial cells from killing by human complement and from apoptosis through activation of a phosphatidylinositide 3-kinase/Akt pathway. Journal of immunology. 2005; 175(3):1903–10.
55. Marshall NA, Galvin KC, Corcoran AM, Boon L, Higgs R, Mills KH. Immunotherapy with PI3K inhibitor and Toll-like receptor agonist induces IFN-gamma+IL-17+ polyfunctional T cells that mediate rejection of murine tumors. Cancer research. 2012; 72(3):581–91.10.1158/0008-5472.CAN-11-0307 [PubMed: 22158905]
56. Wen PY, Lee EQ, Reardon DA, Ligon KL, Alfred Yung WK. Current clinical development of PI3K pathway inhibitors in glioblastoma. Neuro-oncology. 2012; 14(7):819–29.10.1093/neuonc/nos117 [PubMed: 22619466]
57. Oh T, Ivan ME, Sun MZ, Safaee M, Fakurnejad S, Clark AJ, et al. PI3K pathway inhibitors: potential prospects as adjuncts to vaccine immunotherapy for glioblastoma. Immunotherapy. 2014; 6(6):737–53. [PubMed: 25186604]
58. Rauch I, Muller M, Decker T. The regulation of inflammation by interferons and their STATs. Jak-Stat. 2013; 2(1):e23820.10.4161/jkst.23820 [PubMed: 24058799]
59. Herman SE, Gordon AL, Wagner AJ, Heerema NA, Zhao W, Flynn JM, et al. Phosphatidylinositol 3-kinase-delta inhibitor CAL-101 shows promising preclinical activity in chronic lymphocytic leukemia by antagonizing intrinsic and extrinsic cellular survival signals. Blood. 2010; 116(12): 2078–88.10.1182/blood-2010-02-271171 [PubMed: 20522708]
60. Sgorbissa A, Tomasella A, Potu H, Manini I, Brancolini C. Type I IFNs signaling and apoptosis resistance in glioblastoma cells. Apoptosis : an international journal on programmed cell death. 2011; 16(12):1229–44.10.1007/s10495-011-0639-4 [PubMed: 21858676]

61. Sauer S, Bruno L, Hertweck A, Finlay D, Leleu M, Spivakov M, et al. T cell receptor signaling controls Foxp3 expression via PI3K, Akt, and mTOR. Proceedings of the National Academy of Sciences of the United States of America. 2008; 105(22):7797–802.10.1073/pnas.0800928105 [PubMed: 18509048]

62. Humphries W, Wei J, Sampson JH, Heimberger AB. The role of tregs in glioma-mediated immunosuppression: potential target for intervention. Neurosurgery clinics of North America. 2010; 21(1):125–37.10.1016/j.nec.2009.08.012 [PubMed: 19944972]