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Rindopepimut: anti-EGFRvIII peptide vaccine, oncolytic

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SUMMARY

Glioblastoma, the most common primary malignant brain tumor, is among the most difficult cancers to treat. Despite the aggressive standard of care, including surgical removal followed by radiotherapy with concomitant and adjuvant chemotherapy, the often sudden onset, diffuse infiltrating nature and highly malignant features of the lesion result in a median overall survival of < 15 months. Currently employed standard-of-care therapy for glioblastoma is nonspecific, leading to premature withdrawal of treatment due to off-target toxicity. Rindopepimut is a peptide-based vaccine that elicits a potent humoral and cellular immune response specifically against cells expressing EGFRvIII, a rearranged, cell-surface tyrosine kinase receptor present exclusively in glioblastoma and other common neoplasms. Several phase I and phase II clinical trials have demonstrated that vaccination with rindopepimut is safe, well tolerated and produces a highly potent immune response that effectively eradicates EGFRvIII-expressing tumor cells, leading to a 73% increase in survival among patients with newly diagnosed glioblastoma. Furthermore, temozolomide-induced lymphopenia enhances the rindopepimut-induced immune response against EGFRvIII, allowing for enhanced vaccination responses in the context of standard-of-care chemotherapy. Rindopepimut is currently undergoing evaluation in a phase III international trial for newly diagnosed glioblastoma and is under clinical investigation for recurrent glioblastoma and pediatric brain stem gliomas.

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SOURCE

Celldex Therapeutics, Inc. (US).

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Keywords

Malignant brain tumors; Glioblastoma; Radiotherapy; Chemotherapy; Rindopepimut; CDX-110

BACKGROUND

The most frequently encountered primary malignant brain tumor, glioblastoma (GBM), has a five-year survival rate of < 5% (1). Characterized by the World Health Organization (WHO) as a grade IV neoplasm (2), GBM represents a particularly aggressive and difficult to treat neoplasm. The vast majority of GBM cases (> 90%) develop rapidly with a short clinical history (usually < 3 months) and offer no histopathological evidence of a less malignant precursor lesion. Tumors are heterogeneous and consist of diffusely infiltrating, highly malignant, poorly differentiated astrocytic cells. Regions of necrosis and endothelial proliferation, essential diagnostic features, further reflect the aggressiveness of the lesion. The rapid progression, diffuse infiltration, and highly malignant characteristics of this tumor present a significant therapeutic challenge.

Surgery followed by radiotherapy has long been a mainstay of therapy for GBM. Surgical resection plays a critical role in treatment, allowing for alleviation of devastating mass effect-associated symptoms and histopathological diagnosis and characterization. Mounting evidence suggests that more extensive surgical resection accomplished by 5-aminolevulinic acid-derived tumor fluorescence results in a survival benefit (3). Radiotherapy is likewise an indispensable component of treatment, providing a survival benefit versus supportive care alone (4).

Despite previous debate, chemotherapy has also taken a prominent role in therapy for newly diagnosed GBM. Stupp et al. reported the results of a phase III multi-institutional trial in which patients receiving radiotherapy with concomitant and adjuvant temozolomide had a significantly higher 2-year survival rate (26.5%) versus patients receiving radiotherapy alone (10.4%) (5). Temozolomide, an orally active alkylating agent, offers the additional advantage of a favorable toxicity profile compared with older agents. Even with an aggressive multifaceted approach, including extensive surgical resection, state-of-the-art radiotherapy and multiple cycles of temozolomide chemotherapy, the median overall survival (OS) remains < 15 months (6). Alternate therapeutic approaches for the treatment of newly diagnosed GBM face similar limitations. A phase III trial investigating the use of biodegradable carmustine wafers implanted in the resection cavity during surgery resulted in a median survival increase from 11.6 months to 13.9 months versus surgery and radiotherapy alone (7).

Tumors invariably recur with the current standard of treatment. Therapy at the time of recurrence is determined with consideration of previous treatment, performance status, time to relapse and quality of life. In addition to the option of re-employing previously mentioned modalities, bevacizumab, a humanized monoclonal antibody (MAb) that targets vascular endothelial growth factor (VEGF), can be used alone or in combination with irinotecan or other agents. While anti-VEGF therapy is attractive due to the vascular nature of GBM, the

median OS documented in phase II trials is only 40 weeks or less and considerable thromboembolic toxicity can occur (8, 9).

Given the diffuse infiltration of cancer cells, with the currently employed standard of care it is impossible to completely eliminate cells of malignant potential while preserving intermingled, non-transformed tissue. Toxicity resulting from the nonspecific nature of treatment is a critical limitation leading to premature withdrawal of treatment and disease progression. In contrast, immunotherapy represents an exquisitely precise method to eradicate cancer cells. By exploiting endogenous mechanisms, immunotherapy can selectively target and destroy individual cancer cells while preserving intermixed and surrounding normal tissue.

Rindopepimut is an attractive antitumor immunotherapy currently in late-stage clinical development for the treatment of newly diagnosed GBM and in earlier clinical studies for recurrent GBM and pediatric brainstem gliomas. The peptide-based vaccination is capable of eliciting a potent immune response against epidermal growth factor receptor variant III (EGFRvIII), a rearranged, transmembrane tyrosine kinase receptor entirely absent from normal tissue but frequently expressed on the surface of GBM specimens (10) – approximately 31% – and several other common neoplasms (11). Among EGFRvIII-positive GBM samples, 37–86% of cells express the mutated receptor (12), suggesting that the rearranged EGFRvIII protein is translated with a certain level of homogeneity.

The *EGFRvIII* mutation consists of an in-frame deletion of 801 base pairs coding for the extracellular domain of the wild-type receptor. This results in translation of a novel glycine residue at the fusion junction of the rearranged protein. The novel glycine residue and the newfound proximity of normally distant parts of the extracellular domain result in a highly immunogenic, cell-surface, tumor-specific epitope (13).

The resulting EGFRvIII tyrosine kinase receptor is constitutively active, tumorigenic and produces a more aggressive tumor phenotype linked to poor survival (14). In a clinical study investigating the effect of EGFRvIII expression among 196 patients with GBM, expression of EGFRvIII was an independent negative prognostic indicator in patients surviving 1 year or longer (15). EGFRvIII rearrangement enhances cell growth and migration (16, 17), and confers greater resistance to radiation (18) and chemotherapy (19, 20). The mutated tyrosine kinase receptor also enhances growth of neighboring EGFRvIII-negative tumor cells via interleukin-6 (IL-6) family cytokine-mediated paracrine signaling (21). These aggressive characteristics are further propagated by the release of lipid raft-related microvesicles containing EGFRvIII, which can merge with the plasma membranes of EGFRvIII-negative tumor cells, leading to the transfer of a functionally active oncogenic receptor (22). Furthermore, recent studies indicate that EGFRvIII is expressed in glioma stem cell (GSC) lines (23), an important finding given the paradigm that tumor stem cells (TSCs) represent a subpopulation of cancer cells that give rise to all cells in a differentiated tumor (24). Given its importance in the pathobiology of tumors, its exquisite tumor specificity and its clonal expression on the surface of transformed cells, the EGFRvIII rearrangement is an ideal target for antitumor immunotherapy.

Rindopepimut (CDX-110) consists of PEPvIII (LEEKKGNYVVT DHC), a 13-amino-acid peptide that spans the *EGFRvIII* mutation with an additional terminal cysteine, conjugated to keyhole limpet hemocyanin (KLH). KLH, a high-molecular-weight carrier protein, serves as a potent immunogenic substance that safely enhances the production of an immunological response against the conjugated low-molecularweight peptide. Intradermal injection with rindopepimut results in the production of an EGFRvIII-specific humoral response detectable in the cerebrospinal fluid (CSF) and an EGFRvIII-specific delayed-type hypersensitivity (DTH) response. Administration of rindopepimut with granulocyte–macrophage colony-stimulating factor (GM-CSF) further enhances the tumor-specific immune responses, and in numerous preclinical and clinical studies has demonstrated the ability to specifically and safely eliminate EGFRvIII-expressing tumor cells.

PRECLINICAL PHARMACOLOGY

Initial preclinical studies demonstrated antitumor efficacy through the use of unarmed, tumor-specific MABs. In order to test this concept in the context of EGFRvIII-positive tumors, MAb Y10 (IgG_{2a}) and L8A4 (IgG₁) were generated to recognize the tumor-specific extracellular EGFRvIII epitope. Passive administration of both antibodies resulted in tumor growth inhibition and therapeutic efficacy in subcutaneous EGFRvIII-positive murine melanoma models. Only Y10, however, resulted in lasting tumor-free survival after withdrawal of treatment (25). Although systemic administration of the EGFRvIII-specific MAB did not produce an increase in survival when EGFRvIII-expressing tumors were implanted in the brain, direct injection of Y10 into the tumor site increased median survival by 286% on average and resulted in 26% long-term survivors ($n = 117$; $P < 0.001$). In vitro, Y10 produced a highly potent antitumor response, resulting in inhibition of DNA synthesis, decreased cellular proliferation, complement activation and antibody-dependent cell-mediated cytotoxicity. Through depletion studies, the in vivo mechanism of action was found to be dependent on Fc receptors and independent of complement activation, T lymphocytes, granulocytes and natural killer (NK) cells.

The following preclinical EGFRvIII-targeted vaccination strategy exploited the ability of dendritic cells (DCs) to generate potent and specific effector responses in the central nervous system (CNS). DCs were pulsed with rindopepimut and administered systemically to mice bearing EGFRvIII-positive, syngeneic, intracranial tumors. Mice in a control group receiving only phosphate-buffered saline or DCs pulsed with an irrelevant random 14-mer peptide died as a result of the tumor, with a median survival time of 46 and 43 days, respectively, while administration of rindopepimut-pulsed DCs resulted in 63% long-term survivors and a median survival time that had not been reached at the termination of the experiment (300 days). Furthermore, 100% of surviving mice treated with rindopepimut-pulsed DCs survived rechallenge, indicating that the vaccine induced antitumor immunological memory (26).

In light of the results achieved by rindopepimut-pulsed DC vaccination, subsequent preclinical studies investigated the efficacy of rindopepimut vaccination in conjunction with GM-CSF as a pragmatic vaccine that could be advanced more easily to the clinic. In 70% of vaccinated mice challenged with subcutaneous tumor cells, palpable subcutaneous tumors

never developed, and in 30% of the mice where they did develop they were significantly smaller in comparison to the control group ($P < 0.05$), where there were no long-term survivors. In an intracerebral challenge model, vaccination resulted in a $> 173\%$ increase in median survival, with 80% of the mice achieving long-term survival ($P = 0.014$). Results were also promising in an established tumor model where administration of a single vaccination post-challenge resulted in a 26% increase in median survival, with 40% achieving long-term survival ($P = 0.007$). Further mechanistic analysis found NK and CD8+ T cells to be the effector cell population, while passive transfer of sera from immunized mice to nonimmunized mice protected 31% of mice from tumor development ($P < 0.05$) (27). Importantly, downregulated or absent EGFRvIII expression was found upon immunohistochemical analysis of tumors that failed to respond to vaccination, suggesting that antigen escape may be associated with treatment failure.

SAFETY

Several clinical studies have demonstrated that rindopepimut administered intradermally in conjunction with GM-CSF is safe and well tolerated. The vast majority of toxicity consisted of injection-site reactions limited to grade 2 toxicity or lower, including erythema, pruritus and swelling, and was experienced throughout treatment. General fatigue, rash, nausea and pruritus have also been reported. Notably, in a few cases, treatment was discontinued due to toxicity. Some patients experienced hypersensitivity reactions which resolved with antihistamine and corticosteroid treatment. One patient experienced grade 3 toxic epidermal necrolysis, a possible dapsone hypersensitivity syndrome, which resolved within 12 days. In all studies to date, there was no significant evidence of autoimmunity, further demonstrating that EGFRvIII is a safe, tumor-specific target for cancer immunotherapy. Long-term analysis (with monthly treatment for up to 7.3 years in some cases) showed no evidence of cumulative toxicity over time. Safety continues to be assessed in ongoing clinical trials.

CLINICAL STUDIES

The EGFRvIII rearrangement was first established clinically as a safe and potentially effective tumor-specific target for GBM immunotherapy in a clinical trial conducted at Duke University Medical Center (VICTORI) (28). The study evaluated the safety and immunogenicity of autologous DCs pulsed with rindopepimut. While histopathologically confirmed newly diagnosed GBM was required for participation, EGFRvIII expression was not included in the eligibility criteria for the phase I toxicity study. Post-gross tumor resection and completion of standard external beam radiotherapy, 12 study participants received 3 consecutive vaccinations with autologous mature DCs pulsed with rindopepimut. In cohorts of three patients, the DC dose was escalated in order to assess safety and efficacy. With 2-week intervals between dosing, three vaccinations in equal doses were administered to patients 10 cm below the inguinal ligament in the upper thigh. Study participants were then monitored for toxicity and immune response until clinical or radiographic progression. No serious adverse events or allergic reactions were observed in the VICTORI trial; adverse events did not exceed grade 2 toxicity and the maximum feasible dose was reached without dose-limiting toxicity. Furthermore, the majority of patients demonstrated EGFRvIII-

specific immune responses. Overall, in vitro T-cell proliferation in response to PEPvIII was significantly greater post-vaccination.

Given the laborious and technically difficult process of producing autologous DC vaccinations, subsequent clinical investigation pursued the use of direct administration of rindopepimut. A phase II multicenter trial (ACTIVATE) (29) was conducted to test immunogenicity of the vaccination regimen and to estimate progression-free survival (PFS) and OS in vaccinated patients with histopathologically confirmed GBM. Eighteen patients were recruited to the study following gross total resection (> 95%) of EGFRvIII-positive tumors with a Karnofsky performance status (KPS) ≥ 80% and no radiographic evidence of progression following standard-of-care radiation and concurrent temozolomide therapy. Beginning 4 weeks after completion of radiotherapy, three rindopepimut vaccinations were administered biweekly, after which the vaccination was given once a month until radiographic demonstration of tumor progression or death.

Toxicity was minimal and mostly related to injection-site reactions, which never exceeded grade 2 toxicity. While one patient was removed from the study due to a presumed severe allergic reaction to vaccine components, several weeks later a similar event occurred when the patient underwent an unrelated procedure. No symptomatic autoimmune reactions were observed, although one patient experienced toxicity of grade 1 leukoencephalopathy. The patient developed asymptomatic areas of T2 signal hyperintensity which subsequently demonstrated contrast enhancement. Although the asymptomatic regions of T2 hyperintensity persisted, contrast enhancement eventually resolved without treatment (Fig. 1).

The vaccination regimen produced humoral and DTH responses in several patients. Of the 14 patients who had serum samples analyzed for EGFRvIII-specific humoral responses, 6 patients (43%; 95% confidence interval [CI]: 18–71%; $P < 0.001$) had newly positive responses. A CSF sample was obtained from one patient demonstrating a positive serum titer; EGFRvIII-specific antibody was present. These results are highly encouraging given the previous clinical finding that peripherally administered EGFRvIII-specific antibodies effectively penetrate tumors, including gliomas, and that tumor uptake is not dependent on the level of EGFRvIII expression (30).

In order to further assess the vaccination-induced immune response, DTH skin tests with PEPvIII and recall antigens were performed. Although no patients showed a DTH response to PEPvIII prior to vaccination, 3 (18%; 95% CI: 4–43%; binomial proportions $P < 0.001$) of 17 demonstrated a positive rindopepimut response post-vaccination. Among these three patients, one also developed an EGFRvIII-specific humoral response.

The median OS from the time of histopathological diagnosis was greater for patients who demonstrated EGFRvIII-specific humoral or DTH responses. The median OS from the time of histopathological diagnosis for the 6 patients who developed EGFRvIII-specific antibodies was 47.7 months (95% CI: 20.8 to ∞ months) versus only 22.8 months (95% CI: 21.0–34.9 months) for the 8 patients who did not develop an antibody response. Furthermore, the median OS from the time of histopathological diagnosis for the 3 patients

who developed DTH responses to PEPvIII was not reached at 50-month follow-up, while the median OS for the 14 patients who did not develop PEPvIII-specific DTH responses was 23.1 months (95% CI: 21.0–44.1 months). Analysis of the study results in the context of methylated-DNA—protein-cystein methyltransferase (MGMT) promoter methylation status revealed further optimistic results. Given that a methylated MGMT promoter is associated with silencing of the DNA repair enzyme and a longer survival in patients with GBM who receive temozolomide (31), analysis of the study results in the context of MGMT promoter methylation status is important to avoid introducing an unintentional bias. Among 13 patients who had tumor samples tested for MGMT status, 7 (54%) had a methylated MGMT promoter. Surprisingly, vaccinated patients with an unmethylated, active MGMT promoter had a longer PFS and OS than patients with a methylated, silenced MGMT promoter who received temozolomide. This raises the possibility that rindopepimut vaccination may be an effective alternative for patients with MGMT-induced temozolomide resistance.

Following histopathological examination of recurrent tumors ($N = 11$), 82% (95% CI: 48–97%) had lost EGFRvIII expression (Fig. 2), and one of the two EGFRvIII-positive recurrent tumors had $< 1\%$ EGFRvIII-positive cells. Importantly, loss of EGFRvIII expression can be attributed to rindopepimut vaccination and not standard-of-care chemoradiation. In a follow-up analysis of 210 consecutive patients with newly diagnosed GBM, 45 patients were found to have EGFRvIII-expressing tumors. The entire EGFRvIII-positive cohort underwent standard-of-care chemoradiation, while 16 of these patients were also vaccinated with rindopepimut. All 16 patients who were vaccinated lost EGFRvIII expression upon biopsy or resection at recurrence. In contrast, all patients receiving only chemoradiation maintained EGFRvIII expression upon biopsy or resection at recurrence ($n = 15$). The difference remains statistically significant ($P < 0.001$; Fisher's exact test), even with the conservative assumption that patients who were not vaccinated and not biopsied ($n = 14$) lost EGFRvIII expression (32).

In order to further investigate the EGFRvIII-specific immune response induced by rindopepimut vaccination in the context of temozolomide therapy, a second phase II multicenter trial (ACT II) was conducted (33). Although immunotherapeutic approaches can precisely target EGFRvIII-expressing tumor cells, the approach could be inhibited by myelosuppressive chemotherapy like temozolomide, known to provide a survival benefit in GBM. Accordingly, EGFRvIII-specific rindopepimut immunogenicity was assessed in the context of standard- dose temozolomide (200 mg/m² per 5 days) or dose-intensified temozolomide (100 mg/m² per 21 days).

As expected, patients in the standard-dose temozolomide cohort experienced transient grade 2 lymphopenia, while patients in the dose-intensified cohort experienced sustained grade 3 lymphopenia with CD3+ T cell ($P = 0.005$) and B cell counts ($P = 0.004$) significantly reduced. The rindopepimut vaccination regimen and patient selection criteria were similar to that in the ACTIVATE trial, although GMCSF was administered with the vaccination in order to enhance the immune response.

Humoral and DTH EGFRvIII-specific immune responses, while absent prior to vaccination, developed in all patients treated with either the standard-dose or dose-intensified regimen

post-vaccination. Unexpectedly, patients vaccinated in the dose-intensified temozolomide cohort produced humoral ($P = 0.037$) and DTH ($P = 0.036$) immune responses of greater magnitude in comparison to patients in the standard-dose temozolomide cohort (Fig. 3). Although counterintuitive, these findings are consistent with preclinical studies (34, 35) and clinical investigation of adoptive T cell transfer (36), and may be a result of homeostatic cytokines that reduce the activation threshold and induce the proliferation of T cells (37). Toxicity was minimal and there was no significant elevation of markers for autoimmunity. Furthermore, tumor cells expressing EGFRvIII were eradicated in nearly all patients (91.6%; 95% CI: 64–99.8%; $P < 0.0001$). The median PFS (15.2 months; 95% CI: 11.0–18.5 months; $P = 0.024$) and median OS (23.6 months; 95% CI: 18.5–33.1 months; $P = 0.019$) exceeded historical controls matched for participation criteria and adjusted for prognostic factors. In addition to providing evidence of efficacy despite temozolomide-induced lymphopenia, the ACT II study further documented the ability to eradicate EGFRvIII-positive tumor cells without autoimmunity.

Although promising, these results warranted further investigation among a larger cohort of patients. A 31-center phase II study was conducted (ACT III) (38) enrolling 65 patients in the treatment arm with newly diagnosed histopathologically confirmed EGFRvIII-expressing GBM and other eligibility criteria similar to that of the previously described clinical studies. In addition to a treatment regimen consisting of resection and radiotherapy with concomitant and adjuvant temozolomide, patients received inguinal intradermal vaccinations consisting of rindopepimut (500 μ g) and GM-CSF (150 μ g) bimonthly for the first three doses, and then monthly until intolerance or progression. With the exception of two cases, toxicity was limited to grade 1 or grade 2 injection-site reactions and one grade 3 urticarial rash. The two cases of more severe toxicity included a grade 2 hypersensitivity reaction that resolved within 1 hour of antihistamine and corticosteroid administration, and one case of grade 3 toxic epidermal necrolysis which resolved within 12 days of hospital admission. Efficacy was further confirmed in the larger ACT III trial; the median PFS from the time of diagnosis was 12.3 months and the median OS from the time of diagnosis was 24.6 months, time periods significantly higher in comparison with historically matched controls ($P = 0.0063$ and $P < 0.0001$ respectively) (Fig. 4).

Given the clinically demonstrated eradication of EGFRvIII-expressing GBM cells, favorable toxicity profile, and median PFS and OS benefit, rindopepimut is currently under investigation in a phase III study for newly diagnosed GBM (ACT IV). The international, randomized, double-blind trial is currently under way at more than 100 centers and plans to enroll an estimated 440 patients with gross total tumor resection, as well as incomplete resections. Given the absolute tumor specificity of the vaccination-induced immune response, vaccination is likely to be well tolerated among patients where only partial resections are possible. After completion of standard chemoradiation, patients will be randomized to receive either rindopepimut/GM-CSF or control KLH vaccinations in combination with the standard adjuvant temozolomide. While the primary objective will be to confirm that rindopepimut/GM-CSF vaccination improves OS in patients with EGFRvIII-positive GBM who have undergone gross total resection, secondary objectives include

assessment of PFS, safety and tolerability of the vaccination, and health-related quality of life issues.

Rindopepimut is also under clinical investigation for use in EGFRvIII-positive recurrent GBM as a combination therapy with bevacizumab (reACT). The clinical trial is currently under way at approximately 20 centers and plans to enroll an estimated 95 patients. The study will include two groups. The first group will consist of bevacizumab-naïve patients who will be randomized (in a 1:1 ratio) to receive in a blinded fashion either rindopepimut/GM-CSF or control (KLH), in combination with bevacizumab. The second group will consist of patients who have experienced GBM progression while on bevacizumab therapy. In this group, rindopepimut/GM-CSF and bevacizumab will be administered in an open-label fashion. The study primarily seeks to assess antitumor activity of rindopepimut/GM-CSF in adults with relapsed GBM, as measured by PFS at 6 months. Additional objectives include evaluating other measures of antitumor activity (objective response rate, overall PFS and OS), assessing safety and tolerability of rindopepimut/GM-CSF given in combination with bevacizumab, characterizing vaccination-induced EGFRvIII-specific immune responses and determining whether clinical activity parameters correlate with changes in EGFRvIII expression status.

Another more recently developed line of clinical investigation is the use of rindopepimut in pediatric patients for the treatment of diffuse intrinsic pontine gliomas. A phase I study at Stanford University is currently recruiting patients between the ages of 3 and 18 who have clinical and neuroradiographic findings consistent with diffuse intrinsic pontine glioma and who have received conventional radiation therapy. The phase I open-label study primarily seeks to assess the safety of rindopepimut vaccination in this context, but will also provide an initial measure of efficacy.

CONCLUSIONS

Rindopepimut is a promising new therapeutic agent for the treatment of EGFRvIII-expressing gliomas. Vaccinating patients with EGFRvIII-positive GBM is safe, well tolerated and produces immunity against EGFRvIII-expressing cells. More specifically, peripheral administration of rindopepimut/GM-CSF results in the production of EGFRvIII-specific humoral and DTH responses. In patients with GBM, vaccination leads to the elimination of EGFRvIII-positive GBM cells without any associated autoimmunity. Importantly, multiple clinical trials demonstrate a significant mean PFS and OS benefit associated with vaccination. The clinically confirmed finding that a systemically administered vaccination can result in elimination of EGFRvIII-expressing tumor cells in the CNS is intriguing and suggests that the immunoprivilege of the brain may not be as strict as once believed.

Notably, rindopepimut therapy is only appropriate for EGFRvIII-positive tumors, and for EGFRvIII-negative GBM other therapeutic options need to be pursued. Although targeting a single antigen has the benefit of minimizing chances of autoimmunity, given the heterogeneous nature of GBM, elimination of EGFRvIII-expressing tumor cells alone is likely insufficient. Clinical trials for multivalent vaccinations are currently under way and

just as current treatment for GBM entails a multimodal approach, successful management of GBM will most likely rely on a variety of therapeutic modalities. Other immunotherapeutic modalities are likely to have a synergistic effect with rindopepimut therapy, and given that rindopepimut targets a single tumor-specific antigen, adverse effects are likely to be minimized in this context. Accordingly, approximately one-third of newly diagnosed GBM cases are EGFRvIII-positive, and in those cases rindopepimut is an attractive therapeutic option that has thus far demonstrated a significant survival advantage.

The ongoing phase III clinical trial (ACT IV) is necessary to continue to assess the safety and efficacy of rindopepimut/GM-CSF therapy for newly diagnosed GBM. We believe there is a strong likelihood that rindopepimut vaccination for patients with EGFRvIII-positive newly diagnosed GBM will show sufficient safety and efficacy in the phase III randomized trial leading to approval by U.S. and European regulatory agencies. The use of the EGFRvIII-targeted vaccine in adult patients with recurrent glioblastoma and in pediatric patients with diffuse intrinsic pontine glioma is likewise promising and awaits further clinical study. Furthermore, breast cancer (39) and head and neck cancer (40, 41) are other systemic malignancies likely to benefit from rindopepimut vaccination. Choosing treatment for cancer based on the expression of a specific molecule like EGFRvIII is a forerunner of a new paradigm in cancer therapy.

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Figure 1.

Axial magnetic resonance images showing progression of multiple lesions surrounding the corpus callosum. Left: T1-weighted, contrast-enhanced image 8 months from first vaccination showing new enhancing lesions distant from the right frontal tumor cavity. Right: T2-weighted image showing hyperintense lesion in similar distribution. Areas of contrast enhancement have resolved, but areas of T2 hyperintensity have persisted. Reprinted with permission. ©2013 American Society of Oncology. All rights reserved. Sampson, J.H. et al. *Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma*. J Clin Oncol 2010, 28(31): 4722–9.

Figure 2.

Epidermal growth factor receptor (EGFR) and EGFR variant III (EGFRvIII) immunohistochemistry of a patient with glioblastoma multiforme (GBM). Staining with (A) EGFR and (B) EGFRvIII before vaccine. (C) Preservation of EGFR staining but (D) specific loss of EGFRvIII staining at recurrence after vaccination. Reprinted with permission. ©2013 American Society of Oncology. All rights reserved. Sampson, J.H. et al. *Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma*. J Clin Oncol 2010, 28(31): 4722-9.

Figure 3.

Dose-intensified (DI) temozolomide (TMZ) enhances EGFRvIII-specific humoral and cellular immune responses. **(A)** ELISA was used to determine EGFRvIII-specific humoral reactivity. Maximum titer is shown. All patients had developed EGFRvIII-specific humoral immune responses when evaluated at vaccine 8. EGFRvIII-specific antibody titers in the DI cohort increased more over time than those in the standard dose temozolomide (STD) cohort, such that the mean maximum antibody titer was significantly higher in the DI cohort (1:634,982) than in the STD cohort (1:186,521; $P = 0.037$; Wilcoxon–Mann–Whitney test). **(B)** Subjects receiving the DI TMZ regimen for 21 days had vaccine-site delayed-type hypersensitivity (DTH) responses that were higher at all time points than those obtained in the less myelosuppressed patients receiving the 5-day STD TMZ schedule. This was statistically significant when evaluated at vaccine 8 ($P = 0.036$; Wilcoxon–Mann–Whitney test). Sampson, J.H., Aldape, K.D., Archer, G.E. et al. *Greater chemotherapy-induced lymphopenia enhances tumor-specific immune responses that eliminate EGFRvIII-expressing tumor cells in patients with glioblastoma*. *Neuro-Oncology* 2011, 13(3): 324-33, by permission of Oxford University Press.

Figure 4.

Progression-free survival (PFS) and overall survival (OS) for ACTIVATE, ACT II and ACT III clinical trials updated as of November 2012. **(A)** PFS from the time of histological diagnosis for study participants receiving rindopepimut vaccinations compared to historically matched patients treated with standard-of-care therapy. **(B)** OS from the time of histological diagnosis for study participants receiving rindopepimut vaccinations compared to historically matched patients treated with standard-of-care therapy.