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Rindopepimut®: A promising immunotherapeutic for the treatment of glioblastoma multiforme

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Summary

Glioblastoma multiforme (GBM) is the most common and aggressive glial cell-derived primary tumor. Current standard of care for patients with GBM includes maximal tumor resection plus adjuvant radiotherapy and temozolomide chemotherapy, increasing median overall survival to a mere 15 months from diagnosis. Because these therapies are inherently non-specific, there is an increased likelihood of off-target and incomplete effects; therefore, targeted modalities are required for enhanced safety and efficacy. Rindopepimut® is emerging as a safe and potentially effective drug for the treatment of GBM. Rindopepimut consists of a 14-mer peptide that spans the length of EGFRvIII, a mutant variant of EGFR found on ~30% of primary GBM, conjugated to the carrier protein keyhole limpet hemocyanin. Vaccination with Rindopepimut has been shown to specifically eliminate cells expressing EGFRvIII. Phase II clinical trials have suggested that vaccination of newly diagnosed GBM patients with Rindopepimut plus adjuvant GM-CSF results in prolonged progression-free and overall survival with minimal toxicity. This review will outline the development of Rindopepimut, as well as the current status of this vaccine.

Keywords

Glioblastoma; EGFRvIII; Immunotherapy; glioma; CDX-110; Rindopepimut

1. Introduction

Classified by the World Health Organization (WHO) as a grade IV malignant astrocytoma, glioblastoma multiforme (GBM) is the most common type of glial cell-derived primary tumor, accounting for ~54% of all diagnosed gliomas [1]. In the United States alone, there are 10,000 new cases of GBM each year, typically affecting individuals around 60 years of age. GBM is also the most aggressive of the primary malignant brain tumors; even with recent advances in standard of care treatment, the median survival of patients with GBM is

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less than 15 months from diagnosis, with a 5-year survival rate of less than 5% [2]. These statistics underscore the importance for more effective treatments for GBM.

Due to the infiltrative nature of GBM into the normal brain parenchyma, specific therapies that target only cancerous cells are imperative. A lack of tumor specificity is the primary drawback of current standard of care therapies for GBM, which include maximal tumor resection, chemotherapy, and radiation therapy. These modalities are inherently non-specific and, therefore, damaging to the surrounding healthy tissues. For this reason, immunotherapy has become a particularly attractive approach for treating cancer, especially for those that occur within the central nervous system [3]. Several immunological modalities have been examined for anti-tumor efficacy, including adoptive cell-based therapies, tumor vaccines, monoclonal antibodies, and cytokine treatment.

This review will focus on the development and efficacy of the GBM vaccine Rindopepimut® – a proteinaceous immunogen capable of eliciting an immune response against the EGFR mutation EGFRvIII. In preclinical studies Rindopepimut has produced significant increases in median survival, and early clinical studies with Rindopepimut have consistently produced median overall survivals that are unexpectedly long with an excellent safety profile [4]. Rindopepimut is now being investigated in a double-blind, randomized Phase III registration trial. Despite these promising results, tumor relapse of antigen-negative tumor cells after treatment does occur. Therefore, additional research is focused on enhancing the efficacy of Rindopepimut by using combinatorial approaches, such as co-treatment with anti-VEGF antibodies (e.g. bevacizumab).

2. Current Therapies for Glioblastoma Multiforme

High-grade gliomas are heterogeneous cancers that are likely derived from aberrant stem cells [5]. The current standard of care for newly diagnosed GBM patients is maximal surgical tumor resection, followed by adjuvant external beam radiation therapy (RT), and temozolomide chemotherapy [1]. In many cases, complete tumor resection is not practical as it may potentially lead to impairment of neurological function, leading to a poorer quality of life in patients; however, advancements in image-guided surgical modalities have enhanced tumor debridement, while mitigating the damage to surrounding tissues. These techniques include intraoperative and functional magnetic resonance imaging (MRI), cortical mapping, tractography, and fluorescence-guided tumor resection. GBM is unique from most other cancers in that these malignancies rarely metastasize. However, surgical resection of local disease is rarely curative due to the invasive nature of this cancer [6, 7]. In ~95% of cases, recurrent tumors are found within 2 cm of the original tumor [8].

The chemotherapeutic typically used to treat GBM is temozolomide (TMZ) [9]. TMZ is an alkylating agent whose therapeutic benefit is derived from its ability to methylate N-7 or O-6 positions of guanine residues, ultimately leading to cell death. The enzyme encoded by O^6-methylguanine–DNA methyltransferase (MGMT), O^6-alkylguanine DNA alkyltransferase, diminishes the efficacy of TMZ by removing alkyl groups from DNA. Consequently, it has been demonstrated that the silencing of MGMT expression via

methylation of the MGMT promoter contributes to a better prognosis in GBM patients [9, 10].

Nitrosoureas have demonstrated the ability to kill glioma cells *in vitro*; however, the short half-life (~20 minutes) and systemic toxicity of these chemicals limit their clinical use. Alternatively, implantation of carmustine (3-bis (2-chloroethyl 1)-1-nitrosourea)-impregnated polymer wafers (Gliadel® wafers) within the surgical cavity have been used to slowly release the chemotherapeutic agent locally over a 2–3 week period [11]. Phase III trials have shown that Gliadel® wafer implantation, post-resection is a safe and effective method of treating newly diagnosed and recurrent GBMs [12].

Approved by the FDA in 2009, the humanized monoclonal IgG1 antibody bevacizumab (Avastin®) has proven safe for the treatment of GBM [13, 14]. Bevacizumab targets the angiogenic vascular endothelial growth factor (VEGF), which is secreted by highly vascularized GBM [15, 16]. The recent results of the two Phase III randomized clinical trials RTOG 0825 and AVAGlio, which investigated the use of bevacizumab along with the current standard of care therapy in newly diagnosed GBM patients, demonstrated mixed results, however. These studies showed that, although bevacizumab was well tolerated, median overall survival was not significantly increased [17].

Several alternative therapeutics are currently being investigated for the treatment of GBM, though the list is much too long to address in this review. Additional targets include receptor tyrosine kinases (e.g. VEGFR, EGFR, HER2, and PDGFR), signal transduction molecules (e.g. mTOR, PKC, and AKT), and post-translational modifiers (e.g. HDAC and farnesyl transferase) [1]. Conventionally, the therapeutic targeting of biological molecules employs synthetic small molecule ligands or antibodies. Though these modalities have their advantages, small molecule drugs often have undesirable off-target effects, and there is debate as to whether antibodies can readily cross into the brain parenchyma of GBM patients. One approach that alleviates these concerns is adoptive cell therapy with effector T cells. Effector T cells are highly specific, with respect to their immunoprotective effects, and they have the exceptional ability to migrate across the highly-selective brain barriers. Host tumor infiltrating T cells have been identified in GBM tumors, however, they are difficult to isolate and, therefore, do not represent suitable candidates for adoptive T cell therapy [18]. As such, a novel type of transgenic T cell is gaining momentum, in the context of cancer therapy, known as chimeric antigen receptors (CARs). These effector T cells are engineered to express receptors composed of the variable region of a tumor antigen-specific antibody conjugated to various T cell receptor signaling molecules, thereby integrating the benefits of MHC-independent antigen recognition of humoral immunity and the immunoglobulin-independent cytotoxicity of cell-mediated immunity. Compared to a cancer vaccine, CAR therapy grants a more precise and defined immunization strategy, generating an immune response that is more easily monitored since only effector activity must be interrogated [19]. Cancer vaccines, however, are capable of developing a more diverse immune response, with the potential to stimulate both humoral and cell-mediated arms of immunity. Furthermore, vaccines tend to be easier to develop, while the development of CARs requires manipulation of the patients own lymphocytes – a process that is not only laborious but is also expensive. A clinical trial aimed at investigating the safety and feasibility of anti-EGFRvIII CARs is

currently recruiting patients (NCT01454596). Following a lymphodepletive host-conditioning regimen in mice, treatment with anti-EGFRvIII CARs resulted in the elimination of intracerebral EGFRvIII-positive tumor cells [20].

In virtually every instance, tumor relapse following treatment is inevitable in patients with GBM. Currently, there is no established standard of care for GBM that recur after the strategies outlined above. Depending on the circumstance, tumors may be re-resected, followed by RT and/or TMZ-therapy; however, this is not always an option. For this reason, attention has focused on safer, cell-specific modalities, such as immunotherapy.

3. Rationale for Glioma Immunotherapy

The idea that immune cells can detect and eradicate cancerous cells was suggested over a century ago. This concept later developed into the immunosurveillance theory, which proposed that lymphocytes continually surveil tissues searching for cells with foreign antigens, subsequently leading to their elimination. If this is true, one would expect that tumors developed in the context of immunodeficiency would retain immunogenic antigens and, therefore, be immunogenic when xenographed into an immunocompetent environment. Coincidentally, a 2001 study demonstrated that 40% of tumors that developed within T and B cell-deficient $RAG^{-/-}$ mice were eliminated when transplanted into wild type mice [21]. Further supporting the hypothesis that the immune system is capable of eradicating cancerous cells, an increased incidence of cancer is exhibited in immunosuppressed individuals, such as those suffering from immune deficient diseases (e.g. AIDS) or organ transplant recipients [22]. The immunosurveillance model does not, however, explain why persistent tumors develop within immunocompetent organisms. Schreiber, Dunn, and Old accounted for this phenomenon in their immune editing model, which states that neoplasms are sculpted by the adaptive and innate arms of the immune system in the early immunosurveillance phase, naturally selecting for cells capable of evading immunological attack. Features that are potentially selected for include low MHC expression, immunosuppressive molecule production (e.g. TGF-β, PD-L1), loss of immunogenic antigen production, loss of NKG2D ligand expression, and IFN-γ insensitivity – several of which have been identified in GBM. Immunoevasive cells that survive seed what will eventually become a clinically-detectible tumor [22]. Consequently, several therapeutics have been developed that attempt to revive immunological detection of neoplasms. These include adoptive cell-based therapies, vaccines, monoclonal antibodies, checkpoint inhibitors, and cytokine treatment.

Unlike neoplasms that occur at most other areas of the body, malignancies of the CNS were thought to be “immunologically privileged.” The notion that the CNS was free of immunological activity was predicated on the absence of any apparent draining lymphatics within the CNS [23], studies showing that intracranial xenografts exist for extended periods of time within immunocompetent hosts, and the tight-knit nature of the blood-brain (BBB), blood-leptomeningeal (BLMB), and blood-cerebrospinal fluid barriers (BCSFB), which block the passage of solutes that would otherwise be readily transported by the vasculature at alternative locations. Moreover, unlike most other tissues of the body, the brain has a requirement for low immunogenicity. The reason for this is two-fold: the brain parenchyma

has a complex structure that is necessary for its functionality, and the CNS has a limited capacity to regenerate.

It was later demonstrated that the CNS could indeed reject xenografts [24], suggesting that "immune privilege" is not absolute [25]. This was corroborated by molecular characterization of cells within the brain that revealed expression of proteins associated with the innate immune system. The most notable of these cells are the microglia – the CNS-resident macrophages – which express pattern recognitions receptors (PRRs): Toll-like receptors (TLR), Nod-like receptors (NOD), and RIG1-like receptors (RLR). Upon activation of microglia, they transform from a ratified morphology into an amoeboid morphology, enabling them to perform various functions including phagocytosis, neuroprotection, and cytotoxicity [26]. Interestingly, PRRs have also been found on endothelial cells, astrocytes, oligodendrocytes, and even neurons [27, 28], profoundly implicating the role of a formidable innate immune system within the CNS.

Even more intriguing are studies demonstrating adaptive immune activity within the CNS. In general, studies suggest that localization of both naïve and effector lymphocytes to the CNS requires the expression of CNS-tropic adhesion molecules. These molecules include PSGL-1, α4β1 integrin, and LFA-1, which bind to endothelial P-selectin, VCAM-1, and ICAM-1, respectively [29, 30]. Once localized within CNS microvessels, lymphocytes extravasate into the perivascular spaces by migrating through endothelial cells, rather than between them, in a process known as transcellular diapedesis [31, 32]. The perivascular sites act as drainage zones for choroid plexus-derived CSF fluid, as well as CNS parenchyma interstitial fluid, and are home to various antigen presenting cells (APCs), including microglia. It is here that T cells sample the CNS environment through their interaction with resident APCs. Only upon activation are lymphocytes able to infiltrate the CNS parenchymal basement membrane and glia limitans [30, 33–35], a process that has been shown to depend on the focal activity of various matrix metalloproteinases – namely MMP-2 and MMP-9 [36].

Despite our incomplete understanding of neuroimmunology, several CNS-targeted immunotherapies have been developed that demonstrate efficacy for the treatment of high grade gliomas. The immune system is unquestionably active within the CNS, albeit to a much lesser extent compared to most other areas of the body. Thus, in order to enhance the potential of these truly powerful therapeutics, further examination of neurobiological and immunological processes are required.

4. Tumor Associated Antigens & EGFRvIII

Cancer is a result of somatic alterations that confer uninhibited growth potential to cells. Genetic changes that drive tumorigenesis affect such processes as response to growth and anti-growth signals, apoptotic evasion, replicative potential, angiogenesis, tissue invasion, metabolism, immune system evasion, genomic instability, and inflammation [37, 38]. Consequently, they are referred to as "driver" mutations, in contrast to "passenger" mutations, which do not provide a selective growth advantage. One possible downstream effect of mutated proteins is the misregulation of other proteins, leading to the development

of alternative tumor associated antigens. These include proteins that are ectopically expressed (e.g. oncofetal proteins) and those that are aberrantly overexpressed. The drawback of therapeutics targeting these antigens, however, is their potential presence on normal tissues, which could elicit autoimmunity [39]. Due to their necessity for proliferation and unique expression on cancer cells, driver mutations serve as preferential therapeutic targets.

Driver mutations are notoriously difficult to identify due to the heterogeneity amongst tumor samples; that is, driver mutations are not ubiquitous in tumors of the same type. Using computational methods, one study estimated there are 49 missense driver mutations in GBM [40]. Popular candidate proteins are those that contribute to the "hallmarks of cancer," such as growth factor signaling. One such protein is the epidermal growth factor receptor (EGFR), a receptor tyrosine kinase involved in transducing signals that modulate cell proliferation, inhibition of apoptosis, angiogenesis, and cell migration, adhesion, and invasion in response to the binding of ligands including extracellular epidermal growth factor (EGF) and transforming growth factor-alpha (TGF-α). This receptor is often overexpressed in GBM [41]. Additionally, a class III deletion mutation, known as EGFRvIII, has been identified in ~30% of newly diagnosed primary GBM [42].

The gene that encodes EGFRvIII is characterized by the deletion of exons 2–7, resulting in a novel junction between exon 1 and exon 8. This removes amino acids 6–273 from the extracellular, ligand binding domain of EGFR and inserts a glycine not found in the original reading frame [43, 44]. EGFRvIII has been shown to enhance tumorigenicity via low but constitutive ligand-independent signaling [45], promote tumor cell migration [46], and confer protection from RT and TMZ-therapy [47–49]. Cells expressing EGFRvIII can also induce tumorigenicity in neighboring EGFRvIII-negative cells by secreting EGFRvIII-bound oncosomes that incorporate into the plasma membrane of neighboring cells [50]. In light of its oncogenic role and prevalence in GBM, EGFRvIII provides an ideal target for GBM immunotherapy [51].

5. Rindopepimut® (CDX-110)

Rindopepimut is a 14-mer peptide that spans the mutation site of EGFRvIII (PEPvIII: NH_2-Leu-Glu-Glu-Lys-Lys-Gly-Asn-Tyr-Val-Val-Thr-Asp-His-Cyt-COOH) conjugated to the immunogenic carrier protein keyhole limpet hemocyanin (KLH). The name Rindopepimut was determined by the United States Adopted Name Council of the American Medical Association according to the naming convention for peptide immunotherapies, while the drug was licensed to Pfizer in 2008. Since 2010, the license for Rindopepimut has been owned by Celldex Therapeutics.

5.1 Preliminary Studies

Rindopepimut was initially used to generate monoclonal antibodies specific to EGFRvIII [52]. One such IgG2a antibody, Y10, demonstrated the ability to protect mice from tumor growth after subcutaneous challenge with melanoma cells (B16) stably expressing EGFRvIII (B16-EGFRvIII). Y10-mediated tumor protection was shown to be a result of autonomous, complement-mediated, and antibody-dependent cell-mediated cytotoxicity

(ADCC). Moreover, protection was dependent on the Fc receptor and independent of complement, granulocytes, NK cells, and T lymphocytes, as indicated by depletion experiments. Although systemic delivery of Y10 was not able to ameliorate intracerebral (i.c.) challenge with B16-EGFRvIII, direct injection of the antibody into the tumor did increase median survival by 286% and increase long-term survival in 26% of mice [53].

To determine whether an immunological memory against EGFRvIII could be established – a function not afforded by passive immunity – a novel method of tumor-antigen presentation using dendritic cells was adapted based on early studies demonstrating that dendritic cells, pulsed with B16 melanoma cell extract or RNA, were able to generate an adaptive immune response [54, 55]. To mediate EGFRvIII specificity, 1 μg Rindopepimut was pulsed into dendritic cells (Rindopepimut-DCs), and C3H mice were vaccinated intraperitoneally, followed by i.c. challenge with syngeneic K1735-EGFRvIII melanoma cells one week later. Treated mice experienced a ~600% increase in median survival time compared to mice vaccinated with 1 μg Rindopepimut alone or PBS. Rindopepimut-DC vaccinated mice that survived were rechallenged, and all survived. Interestingly, both Rindopepimut-DCs and Rindopepimut alone resulted in a similar IgG1 response; however, in the Rindopepimut-DC vaccinated mice, there was a more dramatic IgG2a response – the same antibody class as Y10 [56].

To evaluate the efficacy of Rindopepimut vaccination in the presence of an adjuvant, C3H mice with established i.c. K1735EGFRvIII tumors were vaccinated with 100 μg Rindopepimut with co-administration of Freund's complete adjuvant. This resulted in a 26% increase in median survival with 40% of mice surviving long-term compared to KLH-vaccinated C3H mice. A clinical relevant modality was also assessed using 100 μg Rindopepimut with co-injection of Freund's incomplete adjuvant plus GM-CSF, which is thought to enhance antigen presentation [57, 58], resulting in a ~60% increase in median survival. Sera from the surviving mice had increased concentrations of anti-PEPvIII IgG1 antibody, and passive transfer of sera into s.c. B16-EGFRvIII challenged mice resulted in tumor protection. Interestingly, depletion of CD8+ T cells in C57BL/6J mice diminished vaccine efficacy, although a cellular immune response was not detectable. This may partially explain the effectiveness of the Rindopepimut vaccine on established i.c. tumors. Immunohistochemical analysis of relapsed tumors indicated that 80% exhibited an outgrowth of EGFRvIII-negative cells, suggesting a cause of treatment failure [59].

5.2 Past Clinical Trials

5.2.1 Phase I - VICTORI—Because of the success of Rindopepimut in preclinical *in vivo* studies, clinical studies were performed to determine safety and efficacy in human subjects. The first of these was a small scale Phase I safety trial performed at Duke University Medical Center, known as VICTORI. Criteria for patient selection included newly diagnosed GBM patients, treated with current standard of care resection/RT/TMZ, over the age of 18, with a KPS of ≥ 80. Eligibility was not dependent on EGFRvIII expression in tumors, however. For this study, 12 patients were vaccinated 3 times in the upper thigh, once every 2 weeks, with PBMC-derived autologous DCs pulsed with 500 μg Rindopepimut and evaluated without therapy until progression was evident. The maximum administered dose

of 1×10^8 Rindopepimut-DCs was well accepted with minimal toxicity. The results indicated that T cells from vaccinated patients underwent antigen-specific proliferation *in vitro*, with T cells from 83.3% of patients responding to PEPvIII and 91.7% responding to KLH. Delayed type hypersensitivity (DTH) skin tests were performed to evaluate the presence of a cellular immune response. In all cases, patients were positive before and after vaccination for tetanus toxoid. No patient was responsive to PEPvIII or KLH prior to vaccination; however, 56% and 100% of patients had a positive response to PEPvIII and KLH post-vaccination, respectively. The median progression free survival (PFS) was 10.2 months, and the median overall survival (OS) was 22.8 months after histological diagnosis [60].

5.2.2 Phase II - ACTIVATE—A Phase II trial was performed at Duke University Medical Center to evaluate the efficacy of Rindopepimut, known as ACTIVATE. In this study, the use of DCs was abandoned due to their expense and difficulty to culture. Instead, 18 patients were vaccinated 3 times in the upper thigh, once every 2 weeks, with 500 μg Rindopepimut with 150 μg GM-CSF as an adjuvant. Vaccinations were administered once a month, thereafter, until evidence of progression or death. Generally, Rindopepimut exhibited low toxicity. Patients were again selected based on their status as a newly diagnosed GBM patient, treated with current standard of care (i.e. gross tumor resection and chemo-radiation therapy), over the age of 18, with a KPS of ≥ 80; however, EGFRvIII-expression was now an inclusion criterion. DTH skin tests indicated that, after vaccination, only 18% of patients had a positive response against PEPvIII. Additionally, humoral responses were evaluated, and 43% had positive responses post vaccination. Although the sample size in this trial is too small to make any significant determinations, patients with positive DTH and humoral responses against PEPvIII did display an increased OS compared to those that were negative for these responses. Median PFS and OS from histological diagnosis for Rindopepimut vaccinated patients was 14.2 and 26.0 months, respectively, compared to 6.4 and 15.2 months, respectively, for matched controls who were contemporaneously-treated according to standard of care at MD Anderson. Upon tumor progression, patients within both the control and experimental cohorts received additional anti-tumor therapies. These treatments include TMZ treatment, protein kinase inhibitors, angiogenesis inhibitors (i.e. anti-VEGF antibody and 2-methoxyestradiol), topoisomerase inhibitors, IL13 infusion, and alternative chemotherapeutic agents (other than TMZ). Among recurrent tumors where pathologic tissue could be obtained, 82% lost all EGFRvIII expression. One of the two recurrent tumors that expressed EGFRvIII exhibited < 1% of total cells staining positive for EGFRvIII. Four Rindopepimut-vaccinated patients survived beyond the completion of this study [61]. At the time of this review, two of these patients are still alive, receiving only a monthly treatment with Rindopepimut plus GM-CSF.

5.2.3 Phase II – ACTII—A counterintuitive result from recent studies suggested that enhanced TMZ-induced lymphopenia could improve antitumor immune responses [62, 63]. To determine whether Rindopepimut efficacy could be enhanced by maintenance TMZ therapy, a Phase II trial, known as ACTII, was conducted to evaluate Rindopepimut vaccination in the context of standard TMZ dosing (STD) and dose intensified (DI) TMZ treatment. Patient selection criteria were similar to that of ACTIVATE: recently diagnosed

GBM patients, have undergone gross tumor resection, have received chemo-radiation therapy, over the age of 18, a KPS of ≥ 80, and exhibit positive EGFRvIII expression. Twenty-two patients were selected for this trial. Rindopepimut was administered, with 150 μg GM-CSF, in the upper thigh on the 21st day of a 28 day TMZ cycle. STD resulted in mostly grade 2 lymphopenia (< 800 cells/μL), and DI treatment resulted in predominantly grade 3 lymphopenia (< 500 cell/μL) by the 6th cycle. Though TMZ treatment diminished both T cell and B cell counts, an unexpected statistical increase in CD4+CD25+FOXP3+ T regulatory cells was witnessed in DI-treated patients. Cell-specific and humoral responses were negative prior to vaccination but were almost all positive post vaccination. Interestingly, antibody titers and DTH responses were significantly increased in DI-treated patients. Because of the small sample size, distinctions could not be made concerning the effects of STD and DI treatment on median PFS and OS; however, the PFS and OS of all vaccinated patients from the time of histological diagnosis was 15.2 and 23.6 months, respectively, compared to 6.4 and 15.2 months, respectively, in matched historical controls from the ACTIVATE trial [63]. Upon tumor progression, most patients received additional anti-tumor therapies including TMZ treatment, repeat resection, protein kinase inhibitors, rapamycin, topoisomerase inhibitors, angiogenesis inhibitors (i.e. anti-VEGF antibody), topoisomerase inhibitors, and alternative chemotherapeutics (other than TMZ).

5.2.4 Phase II – ACTIII—ACT III was a single arm, Phase II trial performed at 31 centers in the United States. Sixty-five newly diagnosed GBM patients with EGFRvIII expression were selected for this trial, regardless of HLA subtype. Additional criteria included ≥ 18 years of age, no progression after gross tumor resection and chemo-radiation therapy, and a KPS score of ≥ 70. Patients were vaccinated in the upper thigh with 500 μg Rindopepimut with 150 μg of the adjuvant GM-CSF and treated with STD maintenance therapy. Vaccinations were administered bimonthly, for the first 3 doses, and then on the 21st day of a 28 day treatment cycle until intolerance, tumor progression, or death. Eighty-five percent of patients developed enhanced antibody titers against EGFRvIII, which increased over time. Cellular responses were also evaluated; however, the results were obfuscated by TMZ-induced lymphopenia. No correlation was found among the various HLA types. Median PFS and OS from histological diagnosis was 12.3 and 24.6 months, respectively, compared to 6.4 and 15.2 months, respectively, in matched historical controls from the ACTIVATE trial [64]. EGFRvIII was eliminated in 4/6 (67%) of tumor samples obtained after > 3 months of therapy (personal communication, Celldex). [63]. Upon tumor progression, most patients received additional anti-tumor therapies including TMZ treatment, repeat resection, angiogenesis inhibitors (i.e. anti-VEGF antibody), alternative chemotherapeutics (other than TMZ), radiation, and other investigational agents.

5.2 Ongoing Clinical Trials

5.2.1 Phase II – ReACT—ReACT is a non-pivotal Phase II trial that is currently enrolling for patients with recurrent EGFRvIII-positive GBM. Criteria for selection are individuals who have relapsed on current standard of care treatment (Group 1) and those who have relapsed while being treated with bevacizumab (Group 2). Approximately, 170 relapsed patients will be selected for this study. Group 1 will be vaccinated with 500 μg Rindopepimut plus GM-CSF or KLH alone, each along with bevacizumab. Group 2 will be

vaccinated with Rindopepimut plus GM-CSF, along with bevacizumab. This trial will be double blinded in a manner that half of the bevacizumab naïve patients will receive Rindopepimut/GM-CSF or KLH. Vaccines will be administered until tumor progression ensues, and patients may be treated with alternative therapeutics accordingly.

5.2.2 Phase III – ACTIV—ACTIV is a Phase III, two-arm registration trial that is currently enrolling for EGFRvIII-positive de novo GBM patients who have received current standard of care treatment and have not progressed following chemo-radiation therapy. This study will be carried out at nearly 200 locations worldwide and will accrue until a total of 374 patients with minimal residual disease have been enrolled. Patients will receive vaccinations with 500 μg Rindopepimut plus GM-CSF or KLH alone, each along with maintenance TMZ treatment. Vaccinations will be administered until tumor progression ensues.

6. Pharmacodynamics

Preclinical and clinical data indicates that vaccination with Rindopepimut results in increased PEPvIII-specific antibodies, suggesting a B cell-mediated humoral response [56, 59, 63–65]. The data on Rindopepimut's ability to elicit a cell-mediated response is less clear. Supporting the idea that cytotoxic T cells are involved in anti-EGFRvIII immunity is preclinical data showing that depletion of CD8+ T cells diminishes Rindopepimut's antitumor efficacy [59] and positive clinical DTH tests showing anti-PEPvIII responses [60, 61, 63]. Further studies are needed to elucidate the pharmacodynamics of Rindopepimut.

7. Safety

Autoimmunity and intracranial inflammation are concerns with any CNS-targeted immunotherapy; however, these were not apparent complications associated with Rindopepimut vaccination. All Rindopepimut vaccines used in preclinical and clinical trials were generally well accepted and typically never exceeded grade 2 toxicity (NCI: Common Toxicity Criteria). The most common adverse effect witnessed in vaccinated patients was a low grade reaction at the site of injection. In very rare cases, grade 3 toxicity was exhibited, thereby resulting in discontinuation of treatment.

8. Limitations

Preclinical and clinical studies have demonstrated that Rindopepimut is able to effectively eradicate EGFRvIII-expressing cells. Long-term efficacy is limited, however, by the outgrowth of cells not expressing EGFRvIII, a phenomenon known as antigen escape [61, 64, 66–68]. This occurrence, which is a result of the inherently heterogeneous nature of tumor cell populations, was unexpected due to EGFRvIII's purported status as a "driver" mutation. Concerns have been raised that the loss of EGFRvIII expression in GBM subsequent to a standard of care plus Rindopepimut treatment regimen could be attributed to the mutagenic properties of chemotherapy and radiation. In response to this, within a group of 45 patients with EGFRvIII-positive GBM, 29 patients who received only gross tumor resection and chemoradiation therapy maintained EGFRvIII expression at tumor recurrence; however, recurrent tumors of the 16 patients treated with the standard of care plus

Rindopepimut displayed no EGFRvIII expression [69]. Despite mosaic expression of EGFRvIII within GBM, long term survival (5+ years post-treatment) has been demonstrated. Although the mechanisms underlying this resilience have not yet been elucidated, two possible explanations are that 1) EGFRvIII-expression is vital in some GBM or 2) epitope spreading bestows protection through the development of immunogenicity towards alternative tumor-specific antigens.

Immune suppression may potentially reduce the efficacy of Rindopepimut in patients with GBM. A characteristic sequelae of GBM is a compromised immune system, as suggested by a significantly higher number of immunosuppressive CD4+CD25+FOXP3+ regulatory T cells within GBM tumors compared to tissue-matched controls [70]. Additionally, active immunosuppression by GBM tumors has been demonstrated through the expression of immunosuppressive factors, including IL-10, TGF-β, VEGF, and prostaglandin E_2, as well as the aberrant expression of the immunosuppressive transcription factor STAT3, a potent inducer of an anti-inflammatory response [65, 71–73].

9. Conclusion & Future Perspectives

Clinical studies have consistently supported the potential of Rindopepimut as a safe and effective immunotherapeutic for the treatment of EGFRvIII-positive GBM. Results of these studies have suggested a potential prolongation of progression-free and overall survival with minimal toxicity. These outcomes coincide with the elimination of EGFRvIII-expressing cells from tumors – an effect that is attributed to the autogenous generation of immune responsiveness towards the unique EGFRvIII epitope.

Despite promising results, Rindopepimut is not without limitations. Efficacy is stifled by the survival, and subsequent outgrowth, of glioma cells that do not express EGFRvIII, suggesting the dispensability of this putative “driver” mutation in GBM. Remarkably, a few patients from early clinical trials are surviving long after initial vaccination. Whether this is a result of the development of a pan-tumor immune response or the necessity of EGFRvIII expression in these GBM remains unclear. Antigen escape could theoretically be mitigated through the targeting of additional GBM-specific antigens. Immune suppression may also limit Rindopepimut efficacy. As such, Rindopepimut efficacy may be augmented through combination therapy with treatments targeting immunosuppressive molecules (e.g. CTLA-4, IL-10, TGF-β).

It is expected that Rindopepimut will perform equally beneficial in the ongoing Phase III trials as it has in early clinical trials. Furthermore, based on its success in early clinical trials, Rindopepimut may also provide benefit for other cancers that express EGFRvIII. The achievement of Rindopepimut as a potent therapeutic for one of the most insidious cancers speaks volumes for the potentiality of immunotherapies as specific and powerful antitumor modalities.

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Executive Summary of Rindopepimut®

Background

- Glioblastoma multiforme (GBM) is the most common and aggressive primary malignant glioma.
- The epidermal growth factor receptor deletion mutation EGFRvIII is found in ~30% GBM.

Current Therapy

- The current standard of care for GBM is maximal tumor resection with adjuvant radiotherapy and temozolomide chemotherapy. Median overall survival with this treatment is ~15 months.
- Additional therapies being investigated for GBM include a carmustine-impregnated wafer (Gliadel® wafers) and an anti-VEGF monoclonal antibody (Avastin®).

Overview of Rindopepimut

- Rindopepimut is a 14-mer peptide spanning mutation site of EGFRvIII, conjugated to the carrier protein keyhole limpet hemocyanin.

Pharmacodynamics

- Rindopepimut vaccination increases EGFRvIII-specific antibody titers in preclinical and clinical studies.
- Some Rindopepimut-vaccinated patients exhibited positive EGFRvIII skin test responses, though data suggesting a cell-mediated response remains inconclusive.
- Vaccination with Rindopepimut results in widespread elimination of EGFRvIII-expressing tumor cells.

Clinical Efficacy

- A Phase I trial (VICTORI) evaluated treatment of newly diagnosed GBM patients with autologous dendritic cells electroporated with 500 μg Rindopepimut, demonstrating that Rindopepimut is a safe and immunogenic vaccine.
- Phase II trials (ACTIVATE, ACTII, and ACTIII) evaluated the safety, immunogenicity, and efficacy of 500 μg Rindopepimut/150 μg GM-CSF in newly diagnosed, EGFRvIII-positive GBM patients. ACTII evaluated vaccine effects in the context of enhanced temozolomide-induced lymphopenia. These studies demonstrated a statistical increase in progression-free and overall survival compared to controls.

Safety and tolerability

- All evaluated Rindopepimut vaccines were generally well-tolerated, and toxicity very rarely exceeded grade 2 based on NCI's Common Toxicity Criteria.

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Table 1

Phase I and II rindopepimut trials.

Study (year)	Trial name	Sample size	Patient eligibility	Treatment groups	Toxicity†	EGFRvIII expression postvaccination	PFS	OS	Ref.
Sampson *et al.* (2009)	VICTORI (Phase I)	12	Newly diagnosed GBM, received standard of care treatment, ≥18 years old, KPS ≥80, negative for pregnancy, immunosuppression and/or infection	Up to 1×10^8 autologous DCs pulsed with 500 μg rindopepimut, three doses, 2 weeks apart, injected id. in inguinal region	≤Grade 2	Not determined, EGFRvIII expression was not a requisite for patient eligibility	Median: 10.2 months (95% CI: 5.7–12.6) after histologic diagnosis	Median: 22.8 months (95% CI: 17.5–29.0) after histologic diagnosis	[60]
Sampson *et al.* (2010)	ACTIVATE (Phase II)	18	Newly diagnosed EGFRvIII-positive GBM, gross tumor resection, received chemoradiation, ≥18 years old, KPS ≥ 80, negative for pregnancy, immunosuppression and/or infection	500 μg rindopepimut plus 150 μg GM-CSF administered biweekly for the first three doses, followed by monthly doses until tumor recurrence, injected id. in inguinal region	≤Grade 3¶	11 recurrent patients' tumors examined: nine exhibited no EGFRvIII expression; of the two that were positive, one exhibited <1% EGFRvIII expression	Median: 14.2 months (95% CI: 9.9–17.6) after histologic diagnosis*	Median: 26 months (95% CI: 21.0–47.7) after histologic diagnosis***	[61]
Sampson *et al.* (2011)	ACTII (Phase II)	22	Newly diagnosed EGFRvIII-positive GBM, gross tumor resection, received chemoradiation, ≥18 years old, KPS ≥80, negative for pregnancy, immunosuppression and/or infection	STD‡ and DI§ TMZ cohorts; 500 μg rindopepimut plus 150 μg GM-CSF administered biweekly for the first three doses, followed by monthly doses until tumor recurrence, injected id. in inguinal region	≤Grade 3#	12 recurrent patients' tumors examined: 11 exhibited no EGFRvIII expression	Median: 15.2 months (95% CI: 11.0–18.5) after histologic diagnosis (all patients)**	Median: 23.6 months (95% CI: 18.5–33.1) after histologic diagnosis (all patients)**	[63]
Lai *et al.* (2011)	ACTIII (Phase II)	65	Newly diagnosed EGFRvIII-positive GBM, gross tumor resection, received chemoradiation, ≥18 years old, KPS ≥80, negative for pregnancy, immunosuppression and/or infection	STD‡ TMZ; 500 μg rindopepimut plus 150 μg GM-CSF administered biweekly for the first three doses, followed by monthly doses until tumor recurrence, injected id. in inguinal region	≤Grade 3††	Six recurrent patients' tumors examined: four exhibited no EGFRvIII expression	Median: 12.3 months after histologic diagnosis**	Median: 24.6 months after histologic diagnosis***	[64]

†National Cancer Institute: Common Toxicity Criteria.

‡STD: 200 mg/m^2 for 5 days of a 28-day cycle.

§DI: 100 mg/m^2 for 21 days of a 28-day cycle.

¶One patient removed from study due to presumed allergic reaction.

#Allergic drug reactions concentrated in DI TMZ cohort

††Two patients removed from the study due to toxicity. Both conditions resolved after discontinuation of treatment.

$p < 0.05$; treatment versus historical controls.

$p < 0.01$; treatment versus historical controls.

$p < 0.001$; treatment versus historical controls.

DC: Dendritic cell; DI: Dose-intensified temozolomide maintenance treatment; EGFRvIII: EGF receptor variant III; GBM: Glioblastoma multiforme; GM-CSF: Granulocyte–macrophage colony-stimulating factor; id.: Intradermally; KPS: Karnofsky performance status; OS: Overall survival; PFS: Progression-free survival; STD: Standard temozolomide maintenance treatment; TMZ: Temozolomide.