Neuro-Oncology
19(11), 1425–1426, 2017 | doi:10.1093/neuonc/nox167

EGFRvIII vaccine in glioblastoma—InACT-IVe or not ReACTive enough?

Michael Platten

Department of Neurology, University Hospital Mannheim, Heidelberg University, Germany and DKTK Clinical Cooperation Unit Brain Tumor Immunology, German Cancer Research Center (DKFZ), Heidelberg. Theodor-Kutzer-Ufer 1-3, 68167 Mannheim, Germany (michael.platten@umm.de)

Variant III of the epidermal growth factor receptor (EGFRvIII) fulfills many criteria of a suitable target for a glioblastoma vaccine. Unlike many tumor-associated self antigens currently targeted in vaccine trials, it is specifically expressed by tumor cells but not in healthy tissue, thus representing a so-called neoantigen. The in-frame deletion of the extracellular domain in EGFRvIII results in the fusion of exons 1 and 8 and thus generates a peptide sequence encompassing the fusion point, which is foreign to the immune system. EGFRvIII is the most common gain-of-function mutation in glioblastoma shown to contribute to the malignant phenotype by complex cytokine network alterations.[1] Preclinical studies have shown immunogenicity and efficacy in several tumor models.[2] In the clinical development the vaccine (rindopepimut) has early on been tested in patients with newly diagnosed glioblastoma to ensure low tumor burden and a sufficient time window to achieve a meaningful immune response. The single-arm ACTIVATE, ACT II, and ACT III trials in patients with newly diagnosed, resected, EGFRvIII+ glioblastoma demonstrated a median survival of 20–22 months, comparing favorably to historical matched controls.[3]

With these data at hand, the stage seemed to be set for the first successful registration trial of a glioblastoma immunotherapy. The double blind, phase III ACT-IV trial is an important study and randomized 745 patients with newly diagnosed, resected, EGFRvIII+ glioblastoma stable after radiochemotherapy to receive rindopepimut or control (keyhole limpet hemocyanin [KLH]) concurrent with standard temozolomide.[4] The trial screened 4652 patients, reflecting the prevalence of EGFRvIII of 20%–25%. The primary endpoint was overall survival (OS) for patients with no or small enhancing tumors (<2 cm^2), operationally defined as minimal residual disease (MRD). This endpoint was chosen under the assumption that MRD improves the starting conditions for any immune intervention otherwise deemed to overcome the suppressive microenvironment of large residual tumors. The trial was negative with a median OS of 20.1 months in the rindopepimut group versus 20.0 months in the control group.[4]

To rationalize why ACT-IV was negative despite robust induction of humoral immune responses, it is imperative to go back to the basic assumptions of targeting EGFRvIII with the immune system:

It was known that this variant is expressed only in a fraction of tumor cells, hence representing a subclonal neoantigen. While it was reasonable to speculate that targeting EGFRvIII can be effective despite its subclonality, since EGFRvIII expression is associated with a more malignant tumor cell phenotype, the loss of EGFRvIII expression in the majority of tumors in the early ACT trials[5] cannot be taken as a firm evidence of biological efficacy and immune-mediated elimination. EGFRvIII expression is spontaneously lost in 50% of glioblastomas upon recurrence even with non-EGFRvIII-targeted approaches.[6] In the ACT-IV trial, EGFRvIII expression was lost in recurrent tumor tissue in 57%–59% independently of the treatment arm, and elimination of EGFRvIII did not correlate with outcome (Weller et al, Lancet Oncol 2017). Hence it is fair to conclude that based on the ACT-IV trial data, we have no evidence (i) that rindopepimut enhances or accelerates the elimination of EGFRvIII-expressing tumor cells and (ii) that even the spontaneous loss of EGFRvIII-expressing tumor cells impacts outcome. Unfortunately, we have no data determining whether rindopepimut with respect to intratumoral immunoreactivity was simply not active enough and whether other vaccine strategies and adjuvants are required to induce an effective immunity. The fact that in the secondary endpoint analysis the 2-year survival rate was increased in the rindopepimut versus control group (30% vs 19%; $P = 0.029$) in the significant (≥2 cm^2) residual disease population (Weller et al, Lancet Oncol 2017) is counterintuitive given the initial trial hypothesis. As there is no robust experimental or clinical evidence for the hypothesis that a large amount of antigen-expressing tumor cells is necessary to mediate or amplify antitumor immunity, this signal cannot be viewed as robust enough to guide future clinical trial concepts.

Particularly in light of the development of T cell–based strategies targeting EGFRvIII including chimeric antigen receptor T cells or bispecific antibodies, it is important to analyze the efferent arm of rindopepimut. While antigen-specific humoral immune responses are often taken as a surrogate parameter

© The Author(s) 2017. Published by Oxford University Press on behalf of the Society for Neuro-Oncology. All rights reserved.
For permissions, please e-mail: journals.permissions@oup.com

for immunogenicity in cancer vaccine trials, it is assumed that therapeutic efficacy of most cancer vaccines is primarily mediated by cellular immune responses, most likely cytotoxic T cells.[7] Some preclinical models have suggested, however, that EGFRvIII-KLH–reactive antibodies are therapeutic by mediating antibody-dependent cellular cytotoxicity (ADCC) and suggested that an antigen-specific T-cell response is dispensable.[8] In line with this, a consistent EGFRvIII-specific T-cell response has not been determined, nor has a consistent major histocompatibility complex class I or II epitope required for priming of CD8+ or CD4+ T cells been defined. The ACT studies would have provided ample opportunity to assess potential antigen-specific cellular T-cell responses. The assumption that vaccine-induced anti-EGFRvIII antibodies mediate efficacy will influence decisions on further development of this approach, particularly with respect to finding combination partners to enhance efficacy.

In the ReACT trial, rindopepimut was combined with the vascular endothelial growth factor (VEGF)–neutralizing antibody bevacizumab (BEV). ReACT was a double-blind, randomized, phase II study which enrolled 73 BEV-naïve patients with EGFRvIII+ glioblastoma recurrent after radiochemotherapy.[9] Patients were randomized to receive BEV plus rindopepimut or BEV with control KLH injections. The primary efficacy endpoint—6-month progression-free survival—was 28% (10/36) for rindopepimut compared with 16% (6/37) for control (P = 0.12). In addition, secondary outcome parameters—OS, duration of response, and corticosteroid requirement—favored the combination group. There is, however, a major caveat with respect to drawing firm conclusions from ReACT. In 80% of the study population, entry into the study was based on EGFRvIII expression in the primary tumor tissue only. Assuming that in 50%–60% of patients EGFRvIII expression is lost in recurrent tumor tissue, a substantial fraction (roughly 40%) of the patients entered into the trial were probably EGFRvIII negative. While this underscores the necessity to enroll recurrent patients based on recent (reresected) tumor tissue, the treatment effect of the EGFRvIII vaccine potential imbalances such as IDH-mutated secondary glioblastomas may result in a considerable bias in the small study population. With this caveat, and keeping in mind the uncertain role of BEV in recurrent glioblastoma after the European Organisation for Research and Treatment of Cancer 26101 trial,[10] the potential mechanism of action of the combination of rindopepimut and BEV should be understood before advancing to further clinical trials. Also, ReACT supports the concept that anti-EGFRvIII antibodies are therapeutic, as demonstrated by positive in vitro tumor cell lysis assays in patients who received rindopepimut in addition to BEV. The hypothesis that BEV improves the efficacy of EGFRvIII-specific antibodies, however, is not easy to rationalize. Normalization of tumor vascularization is unlikely to be key, as this would rather inhibit passive transfer of large molecules such as antibodies to the tumor. Neutralizing VEGF may result in reverting tumor-associated immunosuppression particularly mediated by peripheral dendritic cells or antigen presenting cells and tumor-infiltrating myeloid cells or macrophages/microglial cells. Antibody titers were higher in ReACT compared with ACT-IV, which may be due to TMZ in ACT-IV or by enhancing T-cell priming as a consequence of BEV. Alternatively, or in addition, BEV may shape the glioma microenvironment to create a more immune permissive or even stimulatory environment. With respect to EGFRvIII-specific lytic antibodies, reverting the suppressive phenotype of glioma-infiltrating myeloid cells by BEV may be required to unleash the full potential of ADCC. More preclinical and clinical data, however, are needed to substantiate this hypothesis.

The uncertainties of ReACT and the failure of ACT-IV should not distract us from developing further vaccine trials in glioma patients. However, we ought to learn the lesson that important questions should be addressed in any vaccine trial upfront in order to refine concepts, patient selection, and combination strategies. Answers to these important conceptual questions require innovative, thoroughly designed, scientifically driven clinical trials allowing for the assessment of posttreatment tumor tissue in order to understand the magnitude and nature of an intratumoral immune response as a result of the treatment and to monitor mechanisms of response and resistance in correlation to peripheral immune biomarkers, imaging parameters, and outcome.

References

1. Villa GR, Mischel PS. Old player, new partner: EGFRvIII and cytokine receptor signaling in glioblastoma. *Nat Neurosci*. 2016;19(6):765–767.
2. Choi BD, Archer GE, Mitchell DA, et al. EGFRvIII-targeted vaccination therapy of malignant glioma. *Brain Pathol*. 2009;19(4):713–723.
3. Schuster J, Lai RK, Recht LD, et al. A phase II, multicenter trial of rindopepimut (CDX-110) in newly diagnosed glioblastoma: the ACT III study. *Neuro Oncol*. 2015;17(6):854–861.
4. Weller M, Butowski N, Tran DD, et al. ACT IV trial investigators. Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial. *Lancet Oncol*. 2017;18(10):1373–1385.
5. Sampson JH, Heimberger AB, Archer GE, 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):4722–4729.
6. van den Bent MJ, Gao Y, Kerkhof M, et al. Changes in the EGFR amplification and EGFRvIII expression between paired primary and recurrent glioblastomas. *Neuro Oncol*. 2015;17(7):935–941.
7. Weller M, Roth P, Preusser M, et al. Vaccine-based immunotherapeutic approaches to gliomas and beyond. *Nat Rev Neurol*. 2017;13(6):363–374.
8. Heimberger AB, Crotty LE, Archer GE, et al. Epidermal growth factor receptor vIII peptide vaccination is efficacious against established intracerebral tumors. *Clin Cancer Res*. 2003;9(11):4247–4254.
9. Reardon DA, Shuster J, Tran DD, et al. ReACT: overall survival from a randomized phase II study of rindopepimut (CDX-110) plus bevacizumab in relapsed glioblastoma. *J Clin Oncol*. 2015 May;33(15):2009.
10. Wick W, Gorlia T, Bendszus M, et al. Lomustine and bevacizumab in progressive glioblastoma. *N Engl J Med*. 2017, in press.