Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial

*Michael Weller, Nicholas Butowski, David D Tran, Lawrence D Recht, Michael Lim, Hal Hirte, Lynn Ashby, Laszlo Mechtler, Samuel A Goldlust, Fabio Iwamoto, Jan Drappatz, Donald M O'Rourke, Mark Wong, Mark G Hamilton, Gaetano Finocchiaro, James Perry, Wolfgang Wick, Jennifer Green, Yi He, Christopher D Turner, Michael J Yellin, Tibor Keler, Thomas A Davis, Roger Stupp, and John H Sampson, for the ACT IV trial investigators**

Summary

Background Rindopepimut (also known as CDX-110), a vaccine targeting the *EGFR* deletion mutation EGFRvIII, consists of an EGFRvIII-specific peptide conjugated to keyhole limpet haemocyanin. In the ACT IV study, we aimed to assess whether or not the addition of rindopepimut to standard chemotherapy is able to improve survival in patients with EGFRvIII-positive glioblastoma.

Methods In this randomised, double-blind, phase 3 trial, we recruited patients aged 18 years and older with glioblastoma from 165 hospitals in 22 countries. Eligible patients had newly diagnosed glioblastoma confirmed to express EGFRvIII by central analysis, and had undergone maximal surgical resection and completion of standard chemoradiation without progression. Patients were stratified by European Organisation for Research and Treatment of Cancer recursive partitioning analysis class, *MGMT* promoter methylation, and geographical region, and randomly assigned (1:1) with a prespecified randomisation sequence (block size of four) to receive rindopepimut (500 μg admixed with 150 μg GM-CSF) or control (100 μg keyhole limpet haemocyanin) via monthly intradermal injection until progression or intolerance, concurrent with standard oral temozolomide (150–200 mg/m² for 5 of 28 days) for 6–12 cycles or longer. Patients, investigators, and the trial funder were masked to treatment allocation. The primary endpoint was overall survival in patients with minimal residual disease (MRD; enhancing tumour <2 cm² post-chemoradiation by central review), analysed by modified intention to treat. This trial is registered with ClinicalTrials.gov, number NCT01480479.

Findings Between April 12, 2012, and Dec 15, 2014, 745 patients were enrolled (405 with MRD, 338 with significant residual disease [SRD], and two unevaluable) and randomly assigned to rindopepimut and temozolomide (n=371) or control and temozolomide (n=374). The study was terminated for futility after a preplanned interim analysis. At final analysis, there was no significant difference in overall survival for patients with MRD: median overall survival was 20·1 months (95% CI 18·5–22·1) in the rindopepimut group versus 20·0 months (18·1–21·9) in the control group (HR 1·01, 95% CI 0·79–1·30; p=0·93). The most common grade 3–4 adverse events for all 369 treated patients in the rindopepimut group versus 372 treated patients in the control group were: thrombocytopenia (32 [9%] *vs* 23 [6%]), fatigue (six [2%] *vs* 19 [5%]), brain oedema (eight [2%] *vs* 11 [3%]), seizure (nine [2%] *vs* eight [2%]), and headache (six [2%] *vs* ten [3%]). Serious adverse events included seizure (18 [5%] *vs* 22 [6%]) and brain oedema (seven [2%] *vs* 12 [3%]). 16 deaths in the study were caused by adverse events (nine [4%] in the rindopepimut group and seven [3%] in the control group), of which one—a pulmonary embolism in a 64-year-old male patient after 11 months of treatment—was assessed as potentially related to rindopepimut.

Interpretation Rindopepimut did not increase survival in patients with newly diagnosed glioblastoma. Combination approaches potentially including rindopepimut might be required to show efficacy of immunotherapy in glioblastoma.

Funding Celldex Therapeutics, Inc.

Lancet Oncol 2017; 18: 1373–85

Published Online August 22, 2017 http://dx.doi.org/10.1016/S1470-2045(17)30517-X

See Comment page 1294

*Investigators who participated in this trial are listed in the appendix

Department of Neurology (Prof M Weller MD) **and Department of Oncology** (Prof R Stupp MD), **University Hospital and University of Zurich, Zurich, Switzerland; Department of Neurological Surgery, University of California, San Francisco, CA, USA** (N Butowski MD); **Washington University, St Louis, MO, USA** (D D Tran MD); **Stanford University Medical Center, Palo Alto, CA, USA** (L D Recht MD); **The Johns Hopkins Hospital, Baltimore, MD, USA** (M Lim MD); **Juravinski Cancer Centre, Hamilton, ON, Canada** (H Hirte MD); **Barrow Neurological Institute, Phoenix, AZ, USA** (L Ashby MD); **DENT Neurologic Institute, Buffalo, NY, USA** (L Mechtler, MD); **John Theurer Cancer Center, Hackensack, NJ, USA** (S A Goldlust MD); **Columbia University Medical Center, New York, NY, USA** (F Iwamoto MD); **University of Pittsburgh Medical Center, Pittsburgh, PA, USA** (J Drappatz MD); **Department of Neurosurgery, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA** (D M O'Rourke MD); **Westmead Hospital, Westmead, NSW, Australia** (M Wong MD); **University of Calgary, Department of Clinical Neurosciences, Division of Neurosurgery, Foothills Hospital, Calgary, AB, Canada** (Prof M G Hamilton MD); **Fondazione IRCCS Istituto**

Introduction

Glioblastoma is the most common malignant primary brain tumour in adults. Its annual incidence is more than three per 100 000 people worldwide without major regional variation, and men are affected more frequently than women.[1] The standard of care—maximum feasible surgical resection followed by radiotherapy with concomitant and maintenance temozolomide chemotherapy—generally leads to a median overall survival of about 15 months.[2,3]

The tumour-treating fields device, recently reported to extend survival to 20·5 months, represents an additional treatment option for glioblastoma.[4] Treatment at recurrence, which might include second surgery, re-irradiation, alkylating chemotherapy using nitrosoureas such as lomustine or temozolomide rechallenge, or antiangiogenic therapy using bevacizumab, is less well standardised and has not shown a significant improvement in survival in a randomised trial. Poor prognostic factors include poor performance status, older age, incomplete

Neurologico Carlo Besta, Milan, Italy (G Finocchiaro, MD); **Sunnybrook Health Sciences Centre, Toronto, ON, Canada** (Prof J Perry MD); **The University of Heidelberg and German Cancer Research Center, Heidelberg, Germany** (Prof W Wick MD); **Celldex Therapeutics, Inc, Hampton, NJ, USA** (J Green BS, Y He PhD, C D Turner MD, M J Yellin MD, T Keler PhD, T A Davis MD); **and The Preston Robert Tisch Brain Tumor Center, Duke University Medical Center, Durham, NC, USA** (Prof J H Sampson MD)

Correspondence to: Dr Michael Weller, Department of Neurology, University Hospital and University of Zurich, 8091 Zurich, Switzerland
michael.weller@usz.ch

See Online for appendix

Research in context

Evidence before this study

We searched PubMed for scientific literature published in English before Aug 1, 2011, using the search terms "glioblastoma" and publication type "randomized controlled trial" or "clinical trial, phase III". The standard of care for newly diagnosed glioblastoma established in 2005—maximum feasible surgical resection followed by radiotherapy and temozolomide chemotherapy—is associated with median overall survival of about 15 months. Despite the introduction of several investigational approaches in the subsequent years, no treatment has successfully shown further improvements in survival. The addition of the search term "EGFRvIII" and expansion of our search to any clinical trial did not identify any other agents specifically targeting EGFRvIII. Finally, a search including "glioblastoma", "EGFRvIII", and "survival" produced a few retrospective studies showing similar or worse median and long-term survival for patients whose tumour expressed EGFRvIII. Three previous studies of rindopepimut have been done in patients with newly diagnosed, EGFRvIII-expressing glioblastoma and minimal residual disease (MRD). In these studies, rindopepimut was associated with a strong anti-EGFRvIII humoral immune response, a notable reduction in EGFRvIII expression in available recurrent tumour samples, and a median survival of 20–22 months, as compared with about 12 months for a small matched contemporary dataset and 15 months for the small subset of patients with newly diagnosed EGFRvIII-expressing glioblastoma randomly assigned to receive standard of care treatment in the ACT III study.

Added value of this study

To our knowledge, ACT IV is the first randomised trial to assess the efficacy of an EGFRvIII-targeted treatment for patients with newly diagnosed glioblastoma. Despite the strong anti-EGFRvIII immune response generated in patients, the primary study analysis did not show a survival benefit for patients with MRD who received rindopepimut with temozolomide versus those who received temozolomide alone. We recorded a potential long-term survival benefit in exploratory analyses of a subset of patients with significant residual disease (SRD), which might challenge the view that minimal tumour burden is required for immunotherapy to be effective. Also notable is that patients in the control group fared markedly better than matched control datasets available at the time of study design, suggesting that glioblastoma outcomes have improved since the study was originally designed.

Implications of all the available evidence

Our results question the utility of immunotherapy targeting a single tumour antigen with heterogeneous tumour expression, as well as the optimal setting for evaluation of immunotherapy. Patients with more substantial residual disease expressing the target antigen might experience greater benefit from generation of targeted immunity than those with completely resected disease. Recent data from a randomised, double-blind, phase 2 study in recurrent EGFRvIII-positive glioblastoma (the ReACT study) suggest a prominent treatment effect (overall survival HR 0·53, 95% CI 0·32–0·88; p=0·013) for rindopepimut when combined with standard bevacizumab versus bevacizumab alone. Its combination with temozolomide might compromise an immunological effect, by contrast with bevacizumab. The results of ACT IV also question the predictive value of both historical control datasets (matched patients from non-study databases) and small randomised phase 2 trial datasets (such as ACT III) as a basis for the design of phase 3 studies. These data lend support to further clinical trials that use combination strategies such as immunotherapy with angiogenesis inhibition.

resection, and an unmethylated promoter of the DNA repair gene, O6-methylguanine-DNA methyltransferase (*MGMT*). Novel treatment approaches to glioblastoma are therefore urgently needed, and immunotherapy has now become the major area of clinical research.

The *EGFR* gene is amplified in more than 40% of glioblastomas, and *EGFR* amplification is frequently associated with a deletion mutation affecting exons 2–7, referred to as EGFRvIII or delta-EGFR. EGFRvIII expression occurs in roughly 20–30% of all glioblastomas.[5–7] The potential immunogenicity of the EGFRvIII mutation, first recognised several decades ago, resulted in the development of rindopepimut—a peptide vaccine containing the specific novel aminoacid sequence created by the EGFRvIII deletion mutation conjugated to keyhole limpet haemocyanin. Rindopepimut has been explored in two small single-group phase 2 trials, ACTIVATE[8] and ACT II,[9] as well as a larger phase 2 trial ACT III,[6] which was initially planned as an open-label, randomised phase 3 trial but was converted to a single-group design after near-complete voluntary attrition of the first 16 patients randomly assigned to receive temozolomide alone. In these trials, about 100 patients with EGFRvIII-expressing glioblastoma who had received a gross total resection and had no evidence of progression after radiotherapy with concomitant temozolomide were given rindopepimut alone (ACTIVATE) or rindopepimut with adjuvant temozolomide (ACT II and ACT III). The results of these three trials showed a consistent and encouraging progression-free survival in the range of 15 months from diagnosis and overall survival of 24 months from diagnosis, which compared favourably with contemporary patient cohorts who received standard treatment (appendix p 8). The selection of patients with minimal residual disease (MRD) in all these trials after completion of chemoradiation was based on the assumption that MRD would minimise the tumour-associated immunosuppression typical of glioblastoma.

ACT IV was designed as a pivotal, randomised, placebo-controlled, phase 3 clinical trial to assess whether or not

the addition of rindopepimut to standard temozolomide chemotherapy increased overall survival compared with that for temozolomide alone in patients with newly diagnosed EGFRvIII-expressing glioblastoma.

Methods

Study design and participants

ACT IV was a randomised, double-blind, phase 3 study done at 165 hospitals in 22 countries (appendix p 12). The study was open to men and women aged 18 years and older with newly diagnosed EGFRvIII-expressing glioblastoma. Confirmation of glioblastoma histology and EGFRvIII expression analysis from resected tissue by real-time (RT) PCR were done centrally (LabCorp, Research Triangle Park, NC, USA). Patients must have undergone maximal surgical resection and have completed standard radiotherapy (up to 60 Gy) with concomitant temozolomide (75 mg/m² per day).[2] To be eligible, at least 90% of the planned radiotherapy dose had to be delivered. Patients had to have tumour tissue specimens (paraffin-embedded) from surgical resection available for central pathology review, *MGMT* status determination, and analysis of EGFRvIII status. Exclusion criteria were disease progression during chemoradiation, any additional tumour-specific treatment for glioblastoma, inability to taper corticosteroids to 2 mg of dexamethasone or lower (or equivalent) per day for at least 3 days before randomisation, Eastern Cooperative Oncology Group (ECOG) performance status of 3 or higher in the week before randomisation, diffuse leptomeningeal disease, gliomatosis cerebri, infratentorial disease, active infection, metastatic disease, and immunosuppressive disease.

An independent imaging review committee (BioClinica, Princeton, NJ, USA) assessed post-operative and post-chemoradiation brain MRIs, and retrospectively classified patients as having either MRD (<2 cm² of residual enhancing tumour on post-chemoradiation imaging) or significant residual disease (SRD; ≥2 cm² of residual enhancing tumour on post-chemoradiation imaging).

The study was compliant with the Declaration of Helsinki and Good Clinical Practice guidelines. Ethics approval was obtained at all participating centres and all patients provided written informed consent. The full trial protocol can be found in the appendix.

Randomisation and masking

Eligible patients were stratified by *MGMT* promoter methylation status (methylated *vs* unmethylated *vs* unknown), European Organisation for Research and Treatment of Cancer (EORTC) recursive partitioning analysis class (III *vs* IV *vs* V),[10,11] and geographical region (North America and western Europe *vs* all other regions), and were randomly assigned (1:1) to the treatment groups with a prespecified randomisation sequence with a block size of four. Unblinded pharmacists who were otherwise uninvolved in study conduct obtained randomly assigned treatment assignments and managed study treatment via interactive response technology. Study treatments were prepared in the pharmacy and given to study staff in blinded (label included patient ID and expiry information), pre-loaded syringes. Keyhole limpet haemocyanin was given as a control injection to produce a local reaction similar to that expected with rindopepimut to maintain the treatment blind. Pharmacovigilance staff at the study funder and contract research organisation received treatment assignments for individual patients when necessary to comply with international safety reporting. Pharmacy records were audited by an independent team of contract research organisation staff. The study data monitoring committee and the supportive independent statistical group at the contract research organisation viewed unblinded data. All other study staff, patients, and investigators remained masked to treatment assignments.

Procedures

All patients received standard maintenance temozolomide administered orally at a dose of 150–200 mg/m² on days 1–5 of each 28-day cycle, for 6–12 cycles,[2] or longer if consistent with the local standard of care. Patients randomly assigned to the rindopepimut group also received 500 µg of rindopepimut admixed with 150 µg GM-CSF (Leukine, Sanofi Aventis, Bridgewater, NJ, USA), while the control group received 100 µg keyhole limpet haemocyanin (Biosyn, Carlsbad, CA, USA). Each 0·8 mL dose was given as two to eight separate intradermal injections into the skin of the thigh below the groin. The allowance for between two and eight injections allowed for a smaller volume of individual intradermal injections, potentially reducing patient discomfort and risk of leakage. Experimental treatment was started 7–14 days after completion of standard chemoradiation, and was given as two initial priming doses (on study days 1 and 15), then monthly on day 22 of each temozolomide cycle and continuing after the end of maintenance temozolomide until disease progression or intolerance. Because all toxicities related to rindopepimut vaccination were expected to be immunologically mediated, no adjustment to the dose of double-blind vaccine was allowed; however, dose omission or delay was allowed for toxicity.

Brain MRI scans were done within 14 days after completion of chemoradiation, every 8 weeks for 6 months, every 12 weeks from 6 months through year 2, every 16 weeks from years 2–4, and every 26 weeks thereafter, or until documented disease progression. Tumour response and progression were assessed according to the Response Assessment in Neuro-Oncology (RANO) Working Group criteria,[12] with minor modifications for the purpose of protocol standardisation (appendix p 9). Local investigator assessments guided individual treatment decisions. The retrospective imaging review committee assessment, masked to treatment assignment and investigator assessments, was used for the primary analyses of progression-free survival and objective tumour response.

Safety assessments included monthly physical examination, vital signs, routine laboratory monitoring (haematology on days 1 and 22 of each cycle, and blood chemistry and urinalysis on day 1 of each cycle), and evaluation of adverse events using National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE; version 4.0). The MD Anderson Symptom Inventory Brain Tumor (MDASI-BT) and EORTC Core Quality of Life Questionnaire (QLQ-C30) and Brain Cancer Module (BN20) were completed every month throughout treatment by patients who were fluent in a language in which the questionnaires were validated.

To assess EGFRvIII expression, formalin-fixed paraffin-embedded tumour tissue was analysed centrally (LabCorp, Research Triangle Park, NC, USA). EGFRvIII variant and *EGFR* wild-type status were ascertained with a Taqman-based RT-PCR assay done on an ABI Prism (Applied Biosystems Corporation, Foster City, CA, USA) 7900HT instrument. The fluorescence detected was directly proportional to the amount of RNA present and expressed as cycle threshold. We used a predefined cutoff expressed as delta cycle threshold (cycle threshold of EGFRvIII minus that of *EGFR* wild type) of 11·0 or lower to define a sample as positive for EGFRvIII. The threshold, which was selected as a conservative cutoff to minimise the possibility of including EGFRvIII-negative patients, was defined by the delta cycle threshold values of samples from the ACT III study that showed unambiguous calls of positive (n=9) or negative (n=10) by corroboration of immunohistochemistry and PCR results, confirmed by reproducibility testing and accuracy verification.

To assess *MGMT* promoter methylation status, formalin-fixed paraffin-embedded tumour samples were analysed centrally at LabCorp under licence from MDx Health (Irvine, CA, USA) by methylation-specific PCR, based on previously published methods.[13]

To assess humoral responses to the vaccine, antibody titres were measured by ELISA with microtitre plates directly coated with a 14-aminoacid peptide, which spans the exons 1–8 junction of *EGFR* and is specific for the EGFRvIII mutant.[14] Dilutions of patient plasma were incubated in the plates, and the anti-EGFRvIII antibodies were detected with an Fc-γ-specific goat anti-human F(ab')2 antibody conjugated to horseradish peroxidase (Jackson ImmunoResearch Labs, West Grove, PA, USA) followed by tetra-methylbenzidine substrate. Absorbance was measured at wavelength of 450 nm. Patient samples were screened and positive samples titred against a plate-specific floating cutoff point. We calculated the antibody titre for patient samples as the highest dilution with an optical density value greater than the mean plus three times the standard deviation (SD) of replicate-negative control samples run on the same plate. Because the starting dilution was 1:100, we reported samples that screened negative with titres lower than 1:100. For post-hoc exploratory analyses of survival, patients were retrospectively classified according to the trajectory at which anti-EGFRvIII titres developed with rindopepimut treatment (slow, moderate, or rapid).

Typing of HLA class I alleles (A and B loci) and HLA class II alleles (with a –DR locus) by serology or DNA-PCR was done by an American Society for Histocompatibility and Immunogenetics-accredited laboratory (ClinImmune Labs, Aurora, CO, USA) using buccal swab samples.

Outcomes

The primary endpoint was overall survival (defined as the time from randomisation to death) in patients with newly diagnosed, EGFRvIII-positive glioblastoma and MRD. Patients with SRD formed a second exploratory cohort that had not been included in previous studies; overall survival was assessed in these patients and in the entire patient cohort (all randomised patients) as supportive secondary analyses. Secondary endpoints were progression-free survival (defined as the time from randomisation to disease progression or death, whichever occurred first), the proportion of patients achieving an objective tumour response (a confirmed complete or partial response according to the RANO criteria), health-related quality of life (assessed with the MDASI-BT, QLQ-C30, and BN20), and to further characterise the safety profile and overall immunogenicity of rindopepimut in patients with both MRD and SRD. Correlative endpoints were the specific humoral responses to EGFRvIII and post-treatment EGFRvIII expression status.

Statistical analysis

Patients classified with MRD by the imaging review committee were included in the modified intention-to-treat population for the primary analysis of overall survival. 283 deaths in the MRD population at the time of the final analysis were calculated to provide 80% power to detect a target hazard ratio (HR) of 0·714, which corresponded to a 6-month improvement in median overall survival (from 15 months for the control group to 21 months for the rindopepimut group). The target number of deaths was based on a one-sided log-rank test, overall type I error rate of 0·025, and two planned interim analyses of overall survival for superiority using an O'Brien-Fleming group sequential monitoring plan. Allowing for a 48-month accrual period and 10% attrition rate, a sample size of 374 patients with MRD was expected to result in 283 deaths within 72 months of the first randomly assigned patient. Supportive secondary analyses of overall survival were done for all randomly assigned patients (intention-to-treat analysis). To control the family-wise error rate (the probability of making one or more false discoveries when performing multiple hypotheses tests), study analyses were to proceed according to a fixed sequence procedure in which the primary analysis was completed for the MRD population (modified intention to treat) and all enrolled patients (intention to treat) sequentially,

followed by the secondary endpoint analyses (appendix p 1). According to this analysis plan, each sequential statistical test was to be considered positive only if the previous statistical test was positive. The sample size for patients with SRD was not prospectively defined, and analysis of this population was planned as exploratory. Safety analyses included patients who received at least one dose of study treatment.

Overall survival and progression-free survival, including landmark survival rates at 1, 2, and 3 years, were summarised using the Kaplan-Meier method. For overall survival, patients who were still alive or lost to follow-up at the data analysis cutoff date were right-censored. The censoring date was defined as the patients' date of last contact or data analysis cutoff date (whichever occurred first). Primary inferential comparisons between treatment

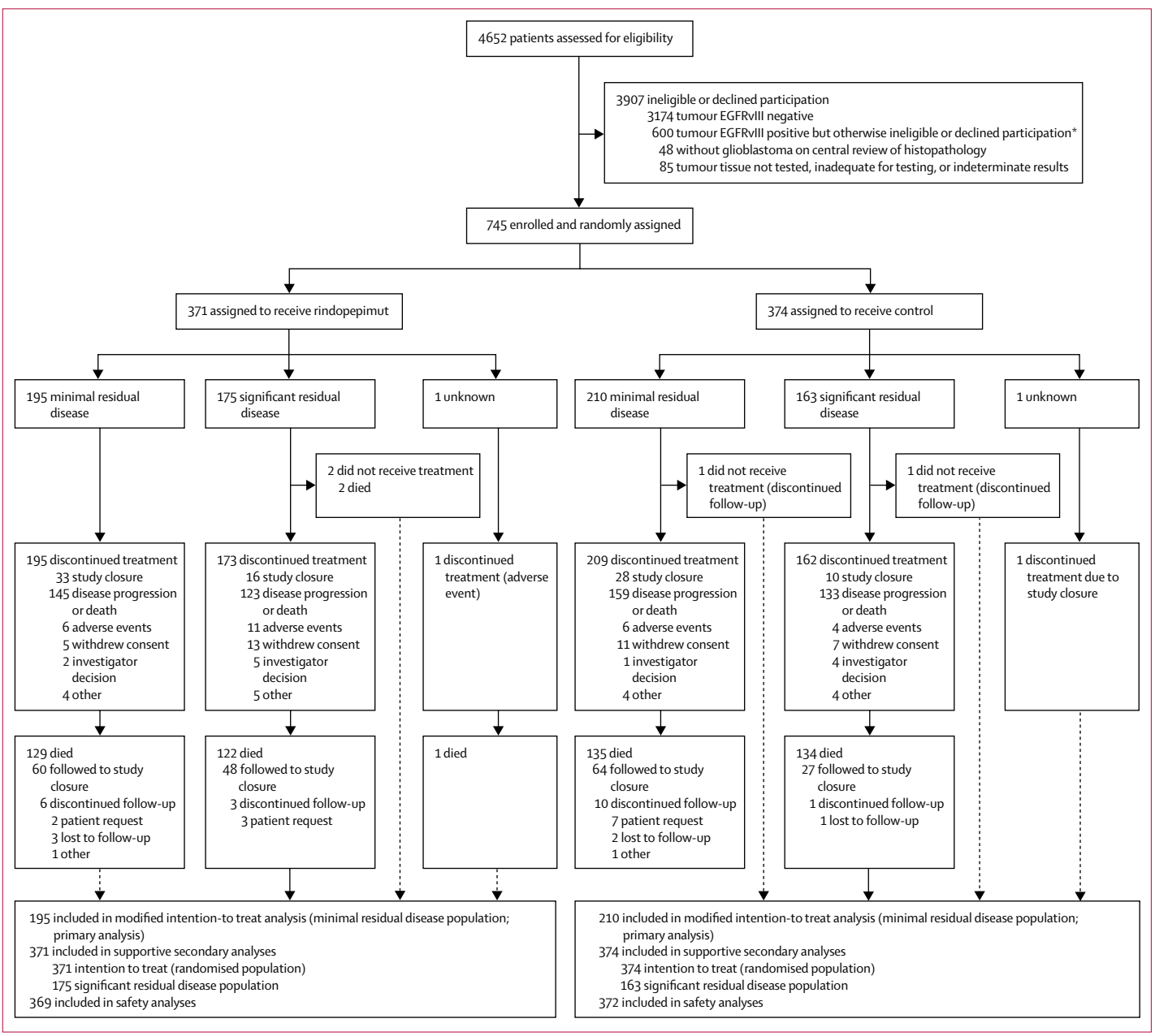

Figure 1: **Trial profile**

*Reasons for failure to enrol were collected via a hard copy screening log, with data for 304 of these 600 patients compiled electronically at the time of study closure. Of these, 143 (47%) declined study participation, 68 (22%) experienced disease progression before study entry, 21 (7%) had a contraindicated concurrent illness or low Eastern Cooperative Oncology Group performance status, 11 (4%) did not receive adequate or standard chemoradiation, 11 (4%) were not candidates for adjuvant temozolomide, ten (3%) were receiving exclusionary doses of corticosteroid, and 40 (13%) were not enrolled for other miscellaneous reasons. GBM=glioblastoma.

groups used the log-rank test stratified by *MGMT* promoter methylation status, adapted recursive partitioning analysis class, and geographical region. HRs were estimated using a stratified Cox proportional hazards model. The proportion of patients who achieved an objective tumour response was summarised for all patients with measurable, enhancing tumour on post-chemoradiation MRI per imaging review committee assessment (ie, the response evaluable population). Subgroup analyses were planned according to age, sex, race, MGMT status, recursive partitioning analysis class, geographical region and baseline tumor burden by investigator assessment (ie, measurable target lesions <2 cm^2 or ≥2 cm^2 per RANO).

For diagnostic purposes, the overall survival proportional hazard assumption was checked by comparing the HRs and assessing the significance level of stratification variables from stratified and unstratified Cox models. We explored the interactions between the treatment group and stratification variables by inclusion of the stratification factors as covariates in the unstratified Cox model. In recognition of the delayed treatment effect previously recorded with other immunotherapies, an exploratory analysis using a weighted log-rank test was also done.[15] In exploratory correlation analyses of HLA type and outcome, the overall survival and progression-free survival hazard ratio was calculated between treatment groups within each HLA type, with p value based on type 3 test from Cox model with treatment group, HLA type, and treatment group * HLA type interaction as covariates.

We planned two interim analyses for superiority and futility after 142 and 212 deaths in the MRD population, representing 50% and 75% of the events required for final analysis. The study was designed with a non-binding approach for efficacy and futility boundaries. Early stopping boundaries for superiority according to an O'Brien-Fleming alpha spending were a p value of 0·002 for the first interim analysis and a p value of 0·018 for the second interim analysis. The futility analyses were based on the observed treatment effect, with HRs of 1·1 or higher and higher than 0·9, respectively, representing boundaries for futility for the two interim analyses. For the first and second interim analyses, respectively, the chances of stopping a positive study for futility were 0·5% and 4·6% and the chances of stopping a negative study for futility were 29% and 78%, respectively. The data monitoring committee evaluated the preplanned interim analyses. SAS version 9.4 was used for all statistical analyses.

This study is registered with ClinicalTrials.gov, number NCT01480479.

	MRD population (primary analysis population)		Intention-to-treat population (all randomly assigned patients)		SRD population	
	Rindopepimut plus temozolomide (n=195)	Control plus temozolomide (n=210)	Rindopepimut plus temozolomide (n=371)	Control plus temozolomide (n=374)	Rindopepimut plus temozolomide (n=175)	Control plus temozolomide (n=163)
Age (years)	59 (51–64)	57 (51–64)	59 (51–64)	58 (52–64)	58 (51–64)	59 (52–64)
Age ≥65 years	46 (24%)	50 (24%)	87 (23%)	87 (23%)	40 (23%)	37 (23%)
Sex						
Male	133 (68%)	121 (58%)	252 (68%)	228 (61%)	118 (67%)	106 (65%)
Female	62 (32%)	89 (42%)	119 (32%)	146 (39%)	57 (33%)	57 (35%)
ECOG performance status						
0	100 (51%)	102 (49%)	165 (45%)	168 (45%)	65 (37%)	65 (40%)
1	86 (44%)	97 (46%)	188 (51%)	185 (50%)	101 (58%)	88 (54%)
2	9 (5%)	11 (5%)	18 (5%)	21 (6%)	9 (5%)	10 (6%)
MGMT promoter status						
Methylated	69 (35%)	73 (35%)	124 (33%)	130 (35%)	55 (31%)	57 (35%)
Unmethylated	107 (55%)	119 (57%)	224 (60%)	218 (58%)	116 (66%)	98 (60%)
Unknown	19 (10%)	18 (9%)	23 (6%)	26 (7%)	4 (2%)	8 (5%)
Recursive partitioning analysis class						
III	25 (13%)	27 (13%)	46 (12%)	37 (10%)	21 (12%)	9 (6%)
IV	139 (71%)	157 (75%)	256 (69%)	274 (73%)	116 (66%)	117 (72%)
V	31 (16%)	26 (12%)	69 (19%)	63 (17%)	38 (22%)	37 (23%)
Time from diagnosis to randomisation (months)	2·8 (2·6–3·1)	2·8 (2·6–3·1)	2·9 (2·6–3·2)	2·8 (2·6–3·1)	2·9 (2·7–3·2)	2·9 (2·7–3·2)
Previous radiotherapy dose (Gy)	60 (60–60)	60 (60–60)	60 (60–60)	60 (60–60)	60 (60–60)	60 (60–60)
Previous temozolomide dose (mg/m^2)	3225 (3150–3300)	3225 (3150– 3375)	3225 (3150–3300)	3225 (3150–3375)	3225 (3150–3375)	3225 (3150–3375)

Data are median (IQR) or n (%). MRD=minimal residual disease. SRD=significant residual disease. ECOG=Eastern Cooperative Oncology Group. *MGMT*=O^6-methylguanine-DNA methyltransferase.

Table 1: **Baseline characteristics**

Role of the funding source
The funder designed the study in collaboration with the investigators, managed the clinical trial database including oversight of data collection, performed statistical analyses, and provided medical writing assistance. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Jan 26, 2012, and Dec 1, 2014, 4652 patients were screened for study eligibility (figure 1). In the cohort of 4519 patients for whom submitted tumour tissue was adequate for EGFRvIII expression analysis, 1345 (30%) had EGFRvIII-expressing tumours. Between April 12, 2012, and Dec 15, 2014, 745 eligible patients with EGFRvIII-expressing tumours were randomly assigned to receive rindopepimut (n=371) or control (n=374). Of these, 405 (195 allocated to rindopepimut and 210 allocated to control) were assigned to the MRD population by central review and included in the primary modified intention-to-treat analysis, and 741 (369 allocated to rindopepimut and 372 allocated to control) received at least one dose of study treatment and were included in the safety analyses. 338 patients (175 allocated to rindopepimut and 163 allocated to control) had SRD and two were unevaluable (one allocated to each group). Pretreatment patient and disease characteristics for randomly assigned patients were well balanced between treatment groups within each analysis population (table 1).

At the second preplanned interim analysis done after 212 deaths had occurred in the MRD population (data cutoff Oct 24, 2015), the futility boundary was crossed. The overall survival HR for rindopepimut versus control in the MRD population was 0·99 (95% CI 0·74–1·31), suggesting that rindopepimut was unlikely to be better than the control in terms of overall survival. Therefore, the study was closed prematurely, and preliminary primary analysis results were released. Additional survival information was obtained as patients were discontinued from the study, and final analyses were done with a data cutoff of April 29, 2016. At study closure and final analysis, 523 deaths (254 in the rindopepimut group [129 in the MRD population] and 269 in the control group [135 in the MRD population]) and 546 progression events (267 in the rindopepimut group [141 in the MRD population] and 279 in the control group [149 in the MRD population]) had occurred.

Overall survival did not differ between the treatment groups for the MRD or intention-to-treat populations (figure 2A, 2B). Median overall survival in the rindopepimut group was 20·1 months (95% CI 18·5–22·1) versus 20·0 months (18·1–21·9) in the control group in the MRD population (HR 1·01, 95% CI 0·79–1·30; p=0·93), and 17·4 months (16·1–19·4) versus 17·4 months (16·2–18·8), respectively, in the intention-to-treat population (HR 0·89, 95% CI 0·75–1·07; p=0·22). In an exploratory analysis of the SRD population (HR 0·79, 95% CI 0·61–1·02; p=0·066), median overall survival was similar between the treatment groups (14·8 months

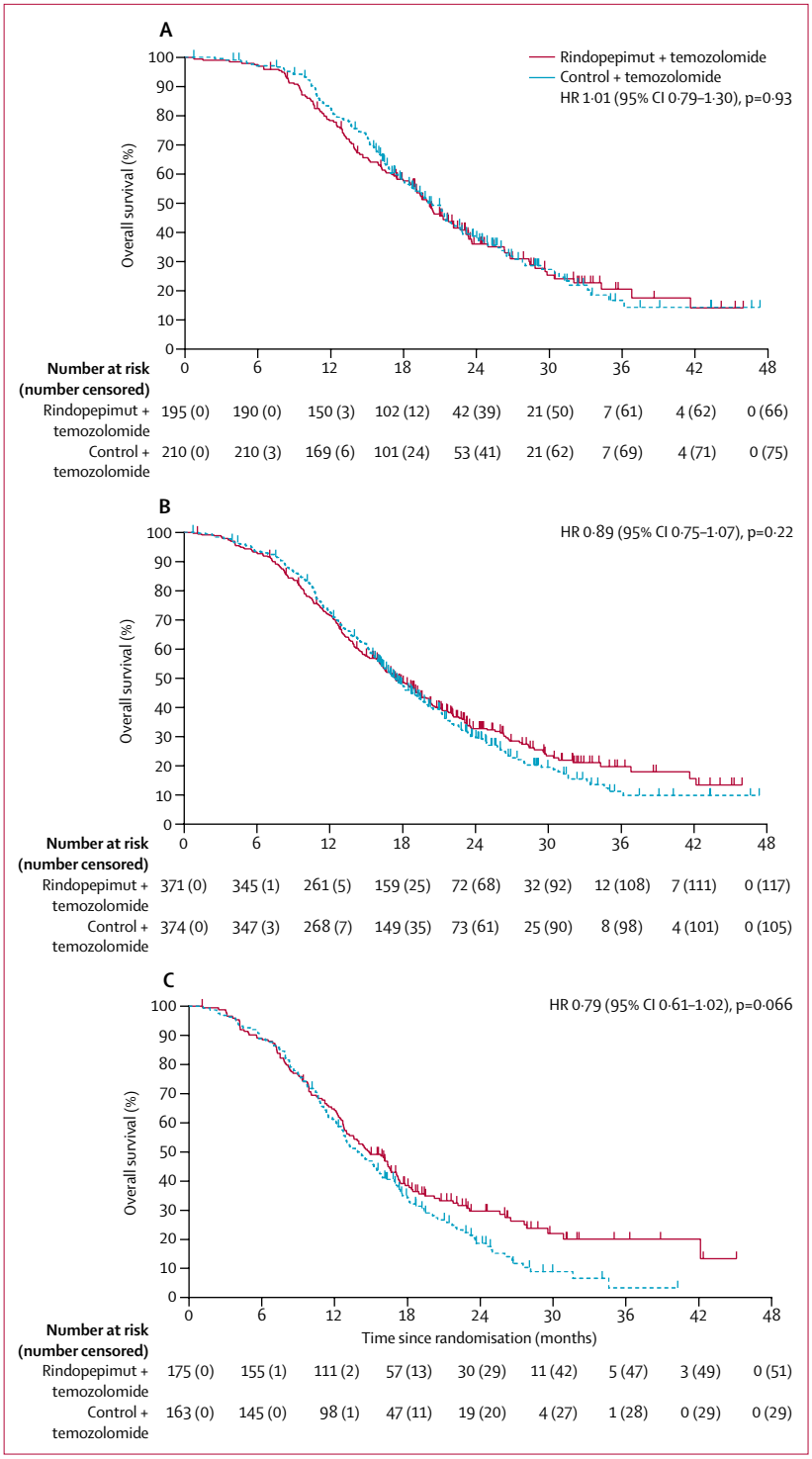

Figure 2: **Overall survival**
Overall survival in (A) the minimal residual disease (primary analysis) population, (B) the intention-to-treat population, and (C) the significant residual disease population. HR=hazard ratio.

[95% CI 12·8–17·1] in the rindopepimut group *vs* 14·1 months [12·6–15·7] in the control group; figure 2C).

In the group of patients who were classified with MRD or SRD by investigator assessment (rather than the central reviewer), overall survival also did not differ between treatment groups (appendix p 2). We also performed prespecified and post-hoc subgroup analyses of overall survival in the MRD and SRD populations; the findings of these subgroup analyses were consistent with our main findings (figure 3).

However, in an exploratory prespecified analysis in the SRD population a difference in long-term survival was recorded, with a 2-year overall survival of 30% (95% CI 23–37) in the rindopepimut group versus 19% (13–26) in the control group (p=0·029). However, this difference was not significant when SRD was classified by investigator assessment rather than by central review (appendix p 2).

On sensitivity analysis of overall survival, we noted some violations of the proportional hazard assumption in various strata (data not shown). We explored interactions between the treatment group and stratification variables and found that none of the interaction terms were significant (data not shown). The weighted log-rank p values for prespecified weights (r1=0, r2=1, 2) were 0·93 and 0·88 for the MRD population, 0·081 and 0·092 for the intention-to-treat population, and 0·021 and 0·017 for the SRD population, respectively.

The median duration of adjuvant temozolomide treatment within the MRD population was 5·2 months (IQR 3·2–10·3) for the rindopepimut group versus 5·0 months (3·0–10·1) for the control group. The median duration of adjuvant temozolomide within the SRD population was 4·1 months (IQR 2·0– 7·3) for the rindopepimut group versus 4·1 months (2·0–6·7) for the control group. Anticancer treatments given in the post-treatment follow-up were well balanced across treatment groups (appendix p 10), although we noted some geographical differences in the type of anticancer treatments used after progression (appendix p 11), with more frequent use of nitrosoureas in the European Union and more frequent use of bevacizumab in the USA.

Progression-free survival was similar between treatment groups within the MRD (median progression-free survival 8·0 months, 95% CI 7·1–8·5, in the rindopepimut group *vs* 7·4 months, 6·0–8·7, in the control group; HR 1·01, 95% CI 0·80–1·29; p=0·91), intention-to-treat (7·1 months, 5·4–7·9 *vs* 5·6 months, 5·1–7·1; 0·94, 0·79–1·13; p=0·51), and SRD (3·7 months, 3·5–5·8 *vs* 3·7 months, 3·3–4·9; 0·86, 0·66–1·12; p=0·28) populations (appendix p 3). The proportion of patients who achieved an objective tumour response in the response-evaluable population irrespective of MRD or SRD status did not differ between treatment groups (31 [15%, 95% CI 10–21] of 208 in the rindopepimut group and 27 [15%, 95% CI 10–21] of 184 in the control group). We noted no significant differences between groups in any of the quality-of-life measures (appendix p 4) or requirement for corticosteroids (which formed a part of response assessment) in the analysis populations (appendix p 5).

Rindopepimut treatment led to robust humoral responses, with treated patients reaching a median peak anti-EGFRvIII antibody titre of 1:25 600 (IQR 3200–204 800). The magnitude of response was similar between the MRD and SRD populations (appendix p 6). The use of corticosteroids did not seem to have a substantial effect on the rindopepimut-induced humoral immune response (appendix p 7). However, we did not record a consistent association between increasingly rapid titre response and clinical outcome (figure 3).

Intensity of tumour EGFRvIII expression (RT-PCR delta cycle threshold in the baseline sample) was not associated with outcome (figure 3). In the small subset of patients with available post-treatment tumour sample, EGFRvIII expression established by RT-PCR was undetectable after treatment for 21 (57%) of 37 patients given rindopepimut and 23 (59%) of 39 patients in the control group. Mean anti-EGFRvIII titre did not differ significantly between the groups of patients with either loss or persistence of tumour EGFRvIII (data not shown). Elimination of EGRFvIII was not associated with with outcome (data not shown).

Correlation analysis of HLA type with outcome was done for the MRD and SRD populations. Although B18, A25, and D11 were each associated with overall survival or progression-free survival in individual analyses, sample sizes were small, this association was not observed consistently in the analysis populations, and these HLA types would not be predicted to bind to the EGFRvIII peptide based on algorithm analysis of the peptide (data not shown).

Rindopepimut was very well tolerated (table 2). Injection-site reactions, consisting mainly of transient grade 1–2 erythema, pruritus, and rash, were reported for most patients who received rindopepimut (294 [80%] of 369), but were also common in the control group (151 [41%] of 372). The most common grade 3–4 adverse events for all 369 treated patients in the rindopepimut group versus 372 patients in the control group were: thrombocytopenia (32 [9%] *vs* 23 [6%]), fatigue (six [2%] *vs* 19 [5%]), brain oedema (eight [2%] *vs* 11 [3%]), seizure (nine [2%] *vs* eight [2%]), and headache (six [2%] *vs* ten [3%]). The most common serious adverse events were seizure (18 [5%] *vs* 22 [6%]) and brain oedema (seven [2%] *vs* 12 [3%]). Of the 523 reported deaths, most (427 [82%]) were due to disease progression (204 [80%] of 254 in the rindopepimut group and 223 [83%] of 269 in the control group), 80 were due to other or unknown causes (41 [16%] in the rindopepimut group and 39 [14%] in the control group), and 16 were due to adverse events (9 [4%] in the rindopepimut group and seven [3%] in the control group). One death—a pulmonary embolism in a

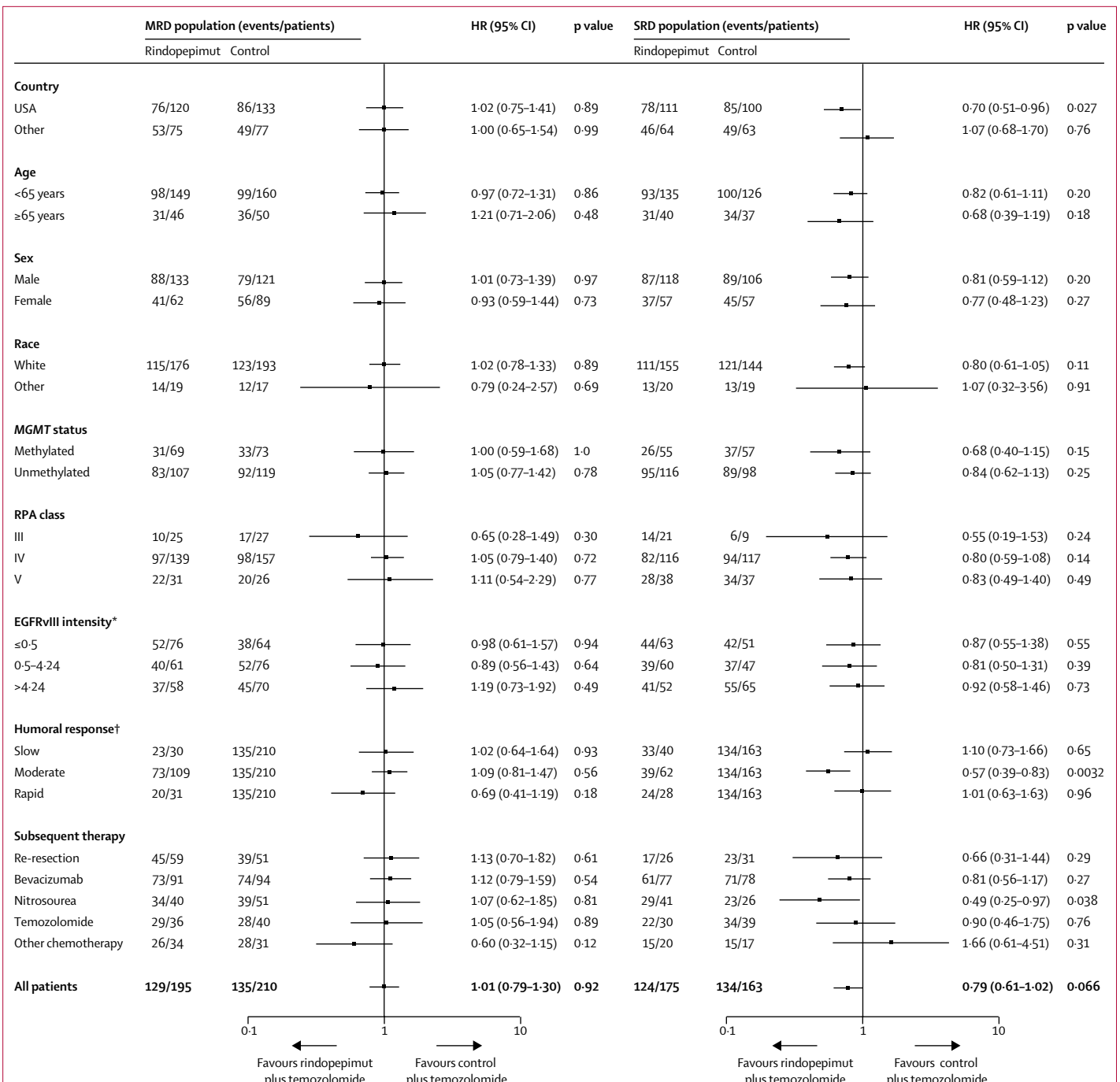

Figure 3: **Subgroup analyses of overall survival**

HR=hazard ratio. MRD=minimal residual disease. SRD=significant residual disease. RPA=recursive partitioning analysis. HRs were calculated for the patients given rindopepimut within each classification versus all patients in the control group. *The fluorescence detected was directly proportional to the amount of RNA present and expressed as cycle threshold. EGFRvIII intensity is defined by delta cycle threshold (cycle threshold of EGFRvIII minus that of *EGFR* wild type). †Humoral response was classified according to the trajectory at which anti-EGFRvIII titres developed with rindopepimut treatment.

64-year-old male patient after 11 months of treatment—was assessed as potentially related to rindopepimut. Eight patients discontinued vaccine treatment due to treatment-related toxic effects. These included two cases of hypersensitivity or allergic reaction, two cases of rash, and single cases of bone or muscle pain and the fatal pulmonary thromboembolism in the rindopepimut group, and single cases of rash and depression in the control group. Despite the finding of hypersensitivity reaction attributed to rindopepimut in previous

studies,[6,8,9] such events were infrequent in both treatment groups in this study (table 2). Similary, no evidence suggested increased toxicities that might theoretically have arisen due to rindopepimut-induced immune infiltration of the brain, such as cerebral oedema or seizure (table 2).

Discussion

The primary analysis of the ACT IV study did not show a survival benefit for patients with EGFRvIII-positive glioblastoma with MRD who received rindopepimut with temozolomide versus those who received a control. Notably, the definition for MRD was increased to less than 2 cm² of residual enhancing tumour in the ACT IV study, as compared with 1 cm² or less in previous studies. Median overall survival from randomisation for patients with MRD given rindopepimut in ACT IV was 20·1 months, which is consistent with the range of 20–22 months recorded in previous uncontrolled trials in the same population.[6,8,9] However, patients in the control group fared substantially better than matched control datasets available at the time of study design (appendix p 8).

	Rindopepimut plus temozolomide (n=369)				Control plus temozolomide (n=372)			
	Grade 1–2	Grade 3	Grade 4	Grade 5	Grade 1–2	Grade 3	Grade 4	Grade 5
Injection-site reaction	294 (80%)	0	0	0	151 (41%)	0	0	0
Fatigue	133 (36%)	6 (2%)	0	0	125 (34%)	19 (5%)	0	0
Nausea	128 (35%)	3 (1%)	0	0	132 (36%)	5 (1%)	0	0
Headache	122 (33%)	5 (1%)	1 (<1%)	0	107 (29%)	10 (3%)	0	0
Thrombocytopenia	66 (18%)	21 (6%)	11 (3%)	0	75 (20%)	14 (4%)	9 (2%)	0
Constipation	86 (23%)	3 (1%)	0	0	91 (25%)	3 (1%)	0	0
Vomiting	75 (20%)	2 (1%)	0	0	76 (20%)	4 (1%)	0	0
Decreased appetite	67 (18%)	4 (1%)	0	0	78 (21%)	1 (<1%)	0	0
Dizziness	56 (15%)	2 (1%)	0	0	69 (19%)	2 (1%)	0	0
Seizure	48 (13%)	7 (2%)	2 (1%)	0	61 (16%)	7 (2%)	1 (<1%)	0
Insomnia	55 (15%)	1 (<1%)	0	0	48 (13%)	0	0	0
Rash	43 (12%)	3 (1%)	0	0	68 (18%)	5 (1%)	0	0
Neutropenia	27 (7%)	12 (3%)	7 (2%)	0	16 (4%)	9 (2%)	8 (2%)	0
Lymphopenia	26 (7%)	17 (5%)	2 (1%)	0	24 (6%)	9 (2%)	3 (1%)	0
Muscular weakness	39 (11%)	5 (1%)	0	0	39 (11%)	12 (3%)	0	0
Diarrhoea	43 (12%)	0	0	0	64 (17%)	0	0	0
Depression	36 (10%)	4 (1%)	0	0	47 (13%)	2 (1%)	1 (<1%)	0
Back pain	35 (10%)	2 (1%)	0	0	38 (10%)	3 (1%)	0	0
Peripheral oedema	33 (9%)	2 (1%)	0	0	43 (12%)	0	0	0
Pruritus	34 (9%)	0	0	0	55 (15%)	1 (<1%)	0	0
Aphasia	28 (8%)	4 (1%)	2 (1%)	0	41 (11%)	11 (3%)	0	0
Anxiety	31 (8%)	1 (<1%)	0	0	44 (12%)	0	0	0
Gait disturbance	28 (8%)	2 (1%)	0	0	36 (10%)	5 (1%)	0	0
Arthralgia	30 (8%)	0	0	0	46 (12%)	1 (<1%)	0	0
Pyrexia	23 (6%)	1 (<1%)	0	0	37 (10%)	0	0	0
Brain oedema	9 (2%)	3 (1%)	5 (1%)	0	9 (2%)	4 (1%)	7 (2%)	0
Hypersensitivity	7 (2%)	1 (<1%)	0	0	5 (1%)	0	0	0
Other nervous system disorders	145 (39%)	33 (9%)	4 (1%)	2 (1%)	175 (47%)	32 (9%)	1 (0.3%)	2 (1%)
Infections and infestations	117 (32%)	19 (5%)	3 (1%)	0	130 (35%)	21 (6%)	2 (1%)	1 (<1%)
Respiratory, thoracic, and mediastinal disorders	89 (24%)	7 (2%)	4 (1%)	3 (1%)	96 (26%)	5 (1%)	3 (1%)	0
Other gastrointestinal disorders	85 (23%)	6 (2%)	1 (<1%)	1 (<1%)	89 (24%)	10 (3%)	0	0
Other musculoskeletal and connective tissue disorders	89 (24%)	3 (1%)	0	0	96 (26%)	8 (2%)	0	0
Other investigations	68 (18%)	14 (4%)	3 (1%)	0	85 (23%)	9 (2%)	3 (1%)	0
Other general disorders and administration-site conditions	73 (20%)	8 (2%)	0	0	80 (22%)	13 (3%)	0	2 (1%)
Injury, poisoning, and procedural complications	67 (18%)	8 (2%)	1 (<1%)	1 (0.3%)	69 (19%)	9 (2%)	1 (<1%)	0
Other psychiatric disorders	64 (17%)	9 (2%)	1 (<1%)	0	64 (17%)	8 (2%)	1 (<1%)	1 (<1%)

(Table 2 continues on next page)

	Rindopepimut plus temozolomide (n=369)				Control plus temozolomide (n=372)			
	Grade 1–2	Grade 3	Grade 4	Grade 5	Grade 1–2	Grade 3	Grade 4	Grade 5
(Continued from previous page)								
Other skin and subcutaneous tissue disorders	72 (20%)	1 (<1%)	1 (<1%)	0	95 (26%)	2 (1%)	0	0
Other metabolism and nutrition disorders	45 (12%)	17 (5%)	3 (1%)	0	56 (15%)	15 (4%)	3 (1%)	0
Eye disorders	60 (16%)	2 (1%)	0	0	70 (19%)	4 (1%)	0	0
Renal and urinary disorders	50 (14%)	6 (2%)	1 (<1%)	0	59 (16%)	5 (1%)	0	0
Vascular disorders	37 (10%)	9 (2%)	4 (1%)	0	40 (11%)	14 (4%)	2 (1%)	0
Other blood and lymphatic system disorders	34 (9%)	6 (2%)	3 (1%)	0	29 (8%)	6 (2%)	2 (1%)	1 (<1%)
Ear and labyrinth disorders	32 (9%)	2 (1%)	0	0	45 (12%)	0	0	0
Cardiac disorders	14 (4%)	3 (1%)	1 (<1%)	1 (<1%)	19 (5%)	0	1 (<1%)	2 (1%)
Reproductive system and breast disorders	16 (4%)	2 (1%)	0	0	14 (4%)	0	0	0
Endocrine disorders	10 (3%)	2 (1%)	0	0	18 (5%)	1 (<1%)	0	0
Neoplasms	11 (3%)	0	0	0	6 (2%)	2 (1%)	1 (<1%)	0
Hepatobiliary disorders	0	1 (<1%)	0	0	3 (1%)	4 (1%)	0	0

Data are grade 1–2 adverse events occurring in ≥10% of patients in either group and all grade 3–5 adverse events in the safety population (all patients who received at least one dose of study treatment irrespective of tumour burden).

Table 2: **Adverse events**

In view of the previous ACT III study in which voluntary attrition of patients randomly assigned to the open-label control group rendered the original randomised design infeasible, we recognised that a truly blinded design would be crucial for ACT IV. Therefore, rather than an inactive placebo, we used keyhole limpet haemocyanin as a control injection to replicate the local reactions (erythema, pruritus, and rash) experienced by nearly all patients who receive rindopepimut. Keyhole limpet haemocyanin can generate immune activation and one might speculate that the better than expected outcome in the control group might result from the generation of an effective immune response unrelated to EGFRvIII. However, it is generally thought that a tumour-specific response triggered by keyhole limpet haemocyanin would require topical application to the tumour itself (intratumoural injection in this case) and that a genuine therapeutic effect is unlikely to result from peripheral intradermal injection. In a small phase 2 trial of patients with recurrent disease (ReACT),[16] the same keyhole limpet haemocyanin control was used with control group outcome no better than expected. The unexpectedly favourable outcome for the ACT IV control group might also have resulted from enrolment of lower risk patients (ie, patients with favourable prognostic factors) compared with previous trials. However, the eligibility criteria and baseline characteristics of patients in the ACT IV study were similar to the previous phase 2 studies of rindopepimut.[6,8,9] Additionally, ACT IV was a large, global phase 3 study, in which outcomes are generally expected to be less favourable than phase 2 studies restricted to specialised centres. Thus, it is more likely that optimisation of standards of care has promoted improvements in outcome over time (appendix p 8).[17]

In view of the negative result for the primary analysis and in accordance with the study's sequential analysis plan, all subsequent analyses should be considered exploratory and cannot be interpreted as conclusive. However, an exploratory analysis showing a potential long-term (2-year) survival benefit in patients with SRD given rindopepimut versus control is an interesting result that does deserve discussion. No imbalances were identified that might have accounted for this signal of differential activity, since baseline prognostic characteristics, corticosteroid dosing, and subsequent therapies were well balanced between both treatment groups. Yet, this apparent treatment effect in the SRD population was less pronounced when tumour burden was defined by the investigator as opposed to central review, and the magnitude of humoral immune response did not consistently correlate with presumed treatment benefit or extent of residual disease. Although further study is needed, this exploratory finding might challenge the view that minimal tumour burden is required for immunotherapy to be most effective.

In the ReACT study,[16] in which patients were not required to have MRD, rindopepimut was associated with a survival advantage (HR 0·53, 95% CI 0·32–0·88; p=0·013) when combined with bevacizumab. Although humoral responses were similar in patients in the ACT IV study irrespective of the amount of residual tumour present, whether effective cellular responses occurred in these patients is unclear. Perhaps residual disease, which would be associated with increased EGFRvIII expression,

is required to generate effective and persistent cellular immunity required for a therapeutic effect. The choice of combination treatment might also account for what seems to be a more prominent treatment effect in the ReACT study than that which might have occurred in the SRD population in ACT IV. VEGF is known to depress tumour immunity and bevacizumab has enhanced immune-mediated antitumour effect in non-clinical models.[18] Alternatively, temozolomide-induced lymphopenia might reduce the efficacy of an immunotherapy. However, this concept is not supported by the ACT II study, in which patients who received dose-intensified temozolomide with rindopepimut developed more robust EGFRvIII-specific humoral immune responses, despite more substantial lymphopenia.

An unexpected finding was the loss of EGFRvIII expression in about 60% of the small subset of patients with tumour tissue available at recurrence, irrespective of study treatment received. Loss of EGFRvIII expression has been reported in most patients given rindopepimut,[6,8,9] but not in those receiving standard-of-care chemoradiation.[19] However, contemporary datasets are contradictory. One RNA-only-based study reported frequent loss of EGFRvIII expression with standard therapy,[20] but this was not confirmed in a recent study by the German Glioma Network.[21] Although screening in ACT IV was done by PCR whereas previous studies used immunohistochemistry, the methods have previously shown good concordance.[6] Supposing that the true rate of EGFRvIII loss at recurrence approaches 50%, one might conclude that the survival benefit recorded in the ReACT trial is derived from only half of the patients, and would therefore probably be quite significant in a cohort with confirmed EGFRvIII expression. More importantly, these data suggest that at least a biopsy is required to verify EGFRvIII positivity before patients with recurrent glioblastoma are enrolled into future trials targeting this mutation. However, the ACT IV results, as well as the lack of stability of EGFRvIII and its expression pattern restricted to subpopulations of tumour cells,[5,21] raise questions about its role as a molecular target for treatment in primary glioblastoma. Trials with other agents in patients with EGFRvIII-mutated tumours might provide additional insight and clarity.

To our knowledge, ACT IV was the most comprehensive study of patients with EGFRvIII-expressing glioblastoma done so far, despite the fact that nearly half of eligible patients declined study participation, probably partly due to the nature of the screening process that allowed for an abbreviated "tissue screening" consent, followed later by the formal study evaluation and consenting process. However, limitations of the study include uncertainties about the significance of the cutoff of EGFRvIII expression for inclusion, and the fact that, because of practical considerations related to patient accrual (that patients would not be identified and screened in time to start study prior to radiation), the vaccine was started after radiotherapy rather than as early as possible. Furthermore, whether concurrent chemotherapy with temozolomide blunts the potential activity of immunotherapy remains controversial. There are some old and some new lessons to be learned from the disappointing outcome of ACT IV. First, even carefully assembled historical controls can be misleading and an unsuitable basis for clinical trial designs, because it is difficult to control for patient selection. Second, even biologically matching data of EGFRvIII loss at progression on anti-EGFRvIII treatment need controls. Third, because the humoral response to EGFRvIII was similar to that recorded in previous studies of rindopepimut, the present study does not support these responses as a reliable predictor of outcome and calls for intensified efforts to establish cellular immune responses as a read-out. Fourth, the selection of one molecular target of immunotherapy might be insufficient, especially if its expression is not stable and not ubiquitous, and multipeptide vaccines against several targets and non-peptides with higher immunogenicity might turn out to be superior. Moreover, the most promising approach seems to be to combine a glioma-specific stimulus based on well defined antigens with a general activation of the immune system. At present, this can most easily be achieved with checkpoint inhibition or with the neutralisation of strong immunosuppressive factors such as TGF-β. Taken together, the results of the ACT IV and ReACT trials support the design of innovative clinical trials evaluating combination approaches to show efficacy of immunotherapy in glioblastoma.

Contributors

MW, JG, TAD, and JHS contributed to the conception and design of the work, data acquisition, and interpretation, and prepared the first draft of the report. NB, DDT, MJY, TK, and RS contributed to the conception and design of the work and data acquisition and interpretation. JP and MGH contributed to the conception and design of the work, operationalisation of the trial in the Canadian Brain Tumor Consortium, and data acquisition and interpretation. LDR, ML, HH, LA, LM, SAG, FI, JD, DMO'R, MW, GF, WW, and CDT contributed to data collection and interpretation; and YH performed data analysis and interpretation. All authors reviewed and revised the manuscript, approved the final version, and agreed on all aspects of the work.

Declaration of interests

MW reports grants and personal fees from Roche, Merck, Actelion, and Novocure; personal fees from MSD, Pfizer, Tocagen, Celldex, Magforce, Bristol-Myers Squibb; and grants from OGD2, Acceleron, and Bayer, outside the submitted work. DDT reports grants from Merck, Novartis, NWBio, Celldex, Stemline, VBL, and TVax; grants and personal fees from Novocure; personal fees from Monteris; and non-financial support from Corvidia, outside the submitted work. ML reports grants from Celldex, during the conduct of the study; grants from Regeneron; grants and personal fees from BMS and Agenus; and personal fees from Merck, Oncorus, Boston Biomedical, Stryker, and Baxter, outside the submitted work. SAG reports personal fees, non-financial support, and is a consultant and on the speaker's bureau and advisory board from Novocure; personal fees and non-financial support from Wex and Bristol-Myers Squibb; and personal fees from Cortice Biosciences, outside the submitted work. FI reports clinical trial costs from Celldex, during the conduct of the study; grants and personal fees from Merck and Novocure; and personal fees from Bristol Myers Squibb, Prime Oncology, Regeneron, AbbVie, and Alexion, outside the submitted work.

DMO'R reports grants from Celldex Therapeutics, Inc, outside the submitted work and has issued patents. MW reports clinical trial costs from Celldex Therapeutics, Inc, during the conduct of the study. MGH reports personal fees from Celldex Therapeutics, Inc, during the conduct of the study. GF reports personal fees from BMS, outside the submitted work. WW reports grants and personal fees from MSD, grants and personal fees from Roche and BMS, outside the submitted work. JG, YH, CDT, MJY, TK, and TAD report personal fees, salary, and equity from Celldex Therapeutics, during the conduct of the study. RS reports fees (to institution) from MSD-Merck & Co, Roche/Genentech, Novartis, Merck KGaA, Darmstadt, AbbVie, and AstraZeneca, outside the submitted work; non-financial support from Novocure Ltd; and their spouse is a full-time employee of Celgene and has equity in Celgene. JHS reports stock from Istari Oncology; stock from Annais; personal fees from Bristol-Myers Squibb; personal fees and consulting and stock options from Immunomic Therapeutics; and patents with royalties paid. NB, LDR, HH, LA, LM, JD, and JP declare no competing interests.

Acknowledgments

We thank the study patients and their families; all the participating investigator and research staff; the additional National Coordinators involved with the study: Myra van Linde (Netherlands), Christine Marosi (Austria), Carmen Balana (Spain), Ahmed Idbaih (France), Tzahala Tzuk-Shina (Israel), and Frank Saran (UK); the Independent Data Monitoring Committee: Richard Kaplan, Alexander Eggermont, David Schiff, Steven Piantadosi, and John Crowley; Jennifer Drescher and Jamye Mart (Celldex Therapeutics, Inc) for their contributions to study management and data collection; and Thomas Hawthorne, Larry Thomas, and Laura Vitale (Celldex Therapeutics, Inc) for performance of correlative studies and analysis of data.

References

1 Weller M, Wick W, Aldape K, et al. Glioma. *Nat Rev Dis Primers* 2015; **1:** 15017.

2 Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med* 2005; **352:** 987–96.

3 Weller M, van den Bent M, Hopkins K, et al. EANO guideline for the diagnosis and treatment of anaplastic gliomas and glioblastoma. *Lancet Oncol* 2014; **15:** e395–403.

4 Stupp R, Taillibert S, Kanner AA, et al. Maintenance therapy with tumor-treating fields plus temozolomide *vs* temozolomide alone for glioblastoma: a randomized clinical trial. *JAMA* 2015; **314:** 2535–43.

5 Weller M, Kaulich K, Hentschel B, et al. Assessment and prognostic significance of the epidermal growth factor receptor vIII mutation in glioblastoma patients treated with concurrent and adjuvant temozolomide radiochemotherapy. *Int J Cancer* 2014; **134:** 2437–47.

6 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:** 854–61.

7 Brennan CW, Verhaak RG, McKenna A, et al. The somatic genomic landscape of glioblastoma. *Cell* 2013; **155:** 462–77.

8 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:** 4722–29.

9 Sampson JH, Aldape KD, Archer GE, et al. Greater chemotherapy-induced lymphopenia enhances tumor-specific immune responses that eliminate EGFRvIII-expressing tumor cells in patients with glioblastoma. *Neuro Oncol* 2011; **13:** 324–33.

10 Mirimanoff RO, Gorlia T, Mason W, et al. Radiotherapy and temozolomide for newly diagnosed glioblastoma: recursive partitioning analysis of the EORTC 26981/22981-NCIC CE3 phase III randomized trial. *J Clin Oncol* 2006; **24:** 2563–69.

11 Curran WJ Jr, Scott CB, Horton J, et al. Recursive partitioning analysis of prognostic factors in three Radiation Therapy Oncology Group malignant glioma trials. *J Natl Cancer Inst* 1993; **85:** 704–10.

12 Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. *J Clin Oncol* 2010; **28:** 1963–72.

13 Hegi ME, Diserens AC, Gorlia T, et al. *MGMT* gene silencing and benefit from temozolomide in glioblastoma. *N Engl J Med* 2005; **352:** 997–1003.

14 Wikstrand CJ, Stanley SD, Humphrey PA, et al. Investigation of a synthetic peptide as immunogen for a variant epidermal growth factor receptor associated with gliomas. *J Neuroimmunol* 1993; **46:** 165–73.

15 Mick R, Chen TT. Statistical Challenges in the design of late-stage cancer immunotherapy studies. *Cancer Immunol Res* 2015; **3:** 1292–98.

16 Reardon DA, Desjardins A, Schuster J, et al. IMCT-08 ReACT: Long-term survival from a randomized phase II study of rindopepimut (CDX-110) plus bevacizumab in relapsed glioblastoma. *Neuro Oncol* 2015; **17:** v109.

17 Gramatzki D, Dehler S, Rushing EJ, et al. Glioblastoma in the Canton of Zurich, Switzerland revisited: 2005 to 2009. *Cancer* 2016; **122:** 2206–15.

18 Johnson BF, Clay TM, Hobeika AC, Lyerly HK, Morse MA. Vascular endothelial growth factor and immunosuppression in cancer: current knowledge and potential for new therapy. *Expert Opin Biol Ther* 2007; **7:** 449–60.

19 Mehta AI, Persson O, Herndon JE, et al. Reply to M.S. Lesniak. *J Clin Oncol* 2011; **29:** 3105–06.

20 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:** 935–41.

21 Weller M, Hentschel B, Kaulich K, et al. *EGFR* gene amplification and variant III (EGFRvIII) mutation in primary and recurrent glioblastoma. *J Clin Oncol* 2016; **34:** 2042.