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Original Article

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Efficacy and Safety of Panitumumab in Patients With *RAF/RAS*-Wild-Type Glioblastoma: Results From the Drug Rediscovery Protocol

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Abstract

Background: The prognosis of malignant primary high-grade brain tumors, predominantly glioblastomas, is poor despite intensive multimodality treatment options. In more than 50% of patients with glioblastomas, potentially targetable mutations are present, including rearrangements, altered splicing, and/or focal amplifications of epidermal growth factor receptor (*EGFR*) by signaling through the *RAF/RAS* pathway. We studied whether treatment with the clinically available anti-*EGFR* monoclonal antibody panitumumab provides clinical benefit for patients with *RAF/RAS*-wild-type (wt) glioblastomas in the Drug Rediscovery Protocol (DRUP).

Methods: Patients with progression of treatment refractory *RAF/RASwt* glioblastoma were included for treatment with panitumumab in DRUP when measurable according to RANO criteria. The primary endpoints of this study are clinical benefit (CB: defined as confirmed objective response [OR] or stable disease [SD] ≥ 16 weeks) and safety. Patients were enrolled using a Simon-like 2-stage model, with 8 patients in stage 1 and up to 24 patients in stage 2 if at least 1 in 8 patients had CB in stage 1.

Results: Between 03-2018 and 02-2022, 24 evaluable patients were treated. CB was observed in 5 patients (21%), including 2 patients with partial response (8.3%) and 3 patients with SD ≥ 16 weeks (12.5%). After median follow-up of 15 months, median progression-free survival and overall survival were 1.7 months (95% CI 1.6-2.1 months) and 4.5 months (95% CI 2.9-8.6 months), respectively. No unexpected toxicities were observed.

Conclusions: Panitumumab treatment provides limited CB in patients with recurrent *RAF/RAS*wt glioblastoma precluding further development of this therapeutic strategy.

Key words: glioblastoma; *RAF/RAS*-wildtype; panitumumab; precision medicine; DRUP trial.

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Implications for Practice

There is an unmet need for new effective treatment options for patients with recurrent glioblastomas. In 57% of patients with glioblastomas, targetable alterations in *EGFR* were found known to signal through the *RAF*/*RAS* pathway. However, targeting *EGFR* failed in patients with glioblastomas previously, potentially due to clinical trial design without target selection of *RAF*/*RAS*-wild-type tumors. In this study, we show that despite selection of patients with *RAF*/*RAS*-wild-type glioblastomas, *EGFR*-targeted therapy (panitumumab) is not effective. Future research should focus on the delivery of drugs through the blood-brain barrier and unravelling resistance mechanisms by broader genomic evaluation.

Introduction

Glioblastomas are the most common primary malignant brain tumors in adults, with an annual incidence of 700 patients per year in the Netherlands.[1] Although aggressive first-line treatment consisting of maximal surgical resection followed by radiation with concomitant and adjuvant temozolomide, the prognosis of patients with glioblastomas remains poor.[2] Five years survival is <5% and almost all glioblastomas locally recur after first-line treatment.[3] Treatment options for patients with recurrent glioblastomas include re-surgery, re-irradiation, systemic therapy, and best supportive care. The role of repeated surgery or radiotherapy is controversial.[4] The recommended second-line systemic therapy for patients with recurrent glioblastomas in the United States is bevacizumab since its approval in 2009 by the Food and Drug Administration (FDA) based on the results of two uncontrolled phase II trials with median progression-free survival (PFS) and median overall survival (OS) of 4.2 and 9.2 months, respectively.[5,6] However, in Europe lomustine is the recommended second-line chemotherapy based on a randomized trial which showed no survival advantage of treatment with lomustine plus bevacizumab over treatment with lomustine alone in patients with progressive glioblastomas.[7] This trial showed a median PFS of lomustine treatment alone in patients with glioblastomas of 1.5 months and a median OS of 8.6 months. Therefore, adequate treatment options for patients with recurrent glioblastomas is lacking, and development of new treatment strategies is warranted.[8,9]

Thus far, various factors may have contributed to the failure of new treatment strategies, including a high degree of tumor heterogeneity, the blood-brain barrier (BBB), and a severely immunosuppressive microenvironment.[10] In 2016, a new World Health Organization (WHO) Classification of Tumors of the Central Nervous System (CNS) was implemented.[11] In this guideline molecular parameters were incorporated into the classification of CNS tumors instead of diagnosis based on histology solely. This implementation in combination with the recent advances in genomic technology with large-scale molecular profiling of glioblastomas have led to a better understanding of their molecular landscape.[12] When studying the genomic landscape of glioblastomas, 57% showed evidence of potential targetable mutations, rearrangements, altered splicing, and/or focal amplifications of epidermal growth factor receptor (*EGFR*).[13] *EGFR*, a receptor tyrosine kinase, was discovered as a proto-oncogene approximately 4 decades ago.[14] Expression of *EGFR variant III* (ie, deletion of exons 2-7 of the *EGFR* gene), the most common *EGFR* mutation in patients with glioblastomas, has only been observed in tumors and not in normal tissue, suggesting that it could be a promising candidate for targeted therapy.[15]

EGFR-targeting antibodies have been clinically approved for the treatment of a wide variety of cancers.[16] So far, efficacy data of clinical trials with anti-*EGFR* antibodies in patients with glioblastomas do not seem to be superior to results of the bevacizumab and lomustine trials.[17-19] A phase II clinical trial in which patients with recurrent glioblastomas were treated with cetuximab in combination with bevacizumab and irinotecan showed a response rate of 26%.[17] The same response rates were found for combination of bevacizumab and irinotecan. A phase II study, in which patients with recurrent glioblastomas were treated with cetuximab alone, reported a clinical benefit rate (CBR) of 35%; 3 out of 55 patients had a partial response (5.5%) and 16 patients had stable disease (29.6%). The median time to progression was 1.9 months and the PFS was shorter than 6 months in the majority of patients (50/55, 91%).[18] Nimotuzumab was evaluated in a randomized phase III trial in which it was added to standard therapy for newly diagnosed glioblastomas.[19] This trial failed to show additional benefit of nimotuzumab combined with standard therapy in this patient group. In all these trials, no correlation between *EGFR* overexpression and response or survival was found. This is in line with earlier research in patients with colorectal cancer revealing that *EGFR* expression is not predictive for response to anti-*EGFR* therapy.[20] However, in other tumor types, especially colorectal cancer, it is well known that mutations in *KRAS*, *NRAS*, and *BRAF* genes are associated with poor outcome following anti-*EGFR* therapy and therefore these mutations could be used to exclude patients from treatment with anti-*EGFR* therapy.

The antibody-drug conjugate Depatux-M, directed against activated *EGFR*, as monotherapy also failed to show benefit for patients with recurrent *EGFR* amplified glioblastomas in a randomized controlled phase II trial. In the INTELLANCE 1 phase III study this antibody-drug conjugate was added to standard chemo-irradiation with temozolomide in patients with newly diagnosed *EGFR* amplified glioblastomas. Due to futility this trial was discontinued after an interim analyses.[21]

Panitumumab is an *EGFR*-targeting antibody approved by the FDA and European Medicines Agency (EMA) for treatment in wild-type (wt) *RAS* (no mutations in either *KRAS* or *NRAS*) metastatic colorectal cancer as first-line therapy in combination with folinic acid, fluorouracil, and oxaliplatin (FOLFOX) or in combination with folinic acid, fluorouracil, and irinotecan (FOLFIRI), in second-line in combination with FOLFIRI for patients who have received first-line fluoropyrimidine-based chemotherapy (excluding irinotecan), or as monotherapy following disease progression after prior treatment with fluoropyrimidine-, oxaliplatin-, and irinotecan-containing chemotherapy.[22]

In the ongoing Drug Rediscovery Protocol (DRUP, NCT02925234), patients with advanced cancer who have exhausted all standard of care options are being treated based on their tumor molecular profile with registered targeted treatments outside their labeled indications, systematically

recording efficacy and safety data.[23] In the present article, we describe treatment outcomes of a completed DRUP cohort, in which patients with treatment refractory *RAF*/*RAS*wt recurrent glioblastomas were treated with panitumumab. In this cohort, patients were included based on the WHO Classification of Tumors of the CNS 2016.[11] In this classification, *IDH*-mutant diffuse astrocytic tumors could be identified as glioblastomas based on histological parameters and therefore included in this cohort. However, in the current WHO Classification of Tumors of the CNS these tumors are considered as astrocytoma's and graded as grade 2, 3, or 4 based on histological and genetic parameters.[24] We do realize that these tumors have a different prognosis and therefore an additional exploratory analysis was performed.

Methods

Study Design

DRUP is an ongoing prospective, multicenter, non-randomized basket and umbrella trial in which patients with advanced or metastatic solid tumors, multiple myeloma, or non-Hodgkin lymphoma, who have exhausted all standard of care options, are being treated based on their tumor molecular profile, with targeted- or immunotherapy outside their registered indications. Patients are enrolled in multiple parallel cohorts, based either on tumor type combined with molecular alteration and study drug (umbrella design) or solely on molecular alteration and study drug in a tumor-agnostic cohort (basket design). Patients enrolled in the cohort "Panitumumab for *RAF*/*RASwt* glioblastomas" received 6 mg/kg panitumumab intravenously (iv) every 2 weeks until occurrence of disease progression or intolerable side effects. Dose reductions were allowed up to a minimum of 3.6 mg/kg every 2 weeks. Patients were enrolled in 9 out of 35 DRUP-participating hospitals in the Netherlands, between March 2018 and January 2022. To date, accrual in other cohorts of DRUP is still ongoing.

DRUP was approved by the Medical Ethical Committee of the Netherlands Cancer Institute in Amsterdam and is conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki's ethical principles for medical research. The study included only adults aged ≥18 years and written informed consent was obtained from all the subjects participating in the study.

This study is registered with ClinicalTrials.gov, number NCT02925234.

Patients

Eligible patients had at least clinical and radiological evidence for refractory *RAF*/*RASwt* recurrent glioblastoma with molecular testing demonstrating no mutations in either *BRAF*, *KRAS*, or *NRAS* and stable or decreasing dosage of steroids for at least 7 days prior to the baseline magnetic resonance imaging (MRI). Molecular tests were performed before inclusion within DRUP and therefore not included in this trial. All molecular tests for *BRAF*, *KRAS*, or *NRAS* mutations were accepted, performed on new biopsies, or archived tumor material obtained from primary resection material.

Patients had progressive measurable disease according to Response Assessment in Neuro-Oncology (RANO[25]) and an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2. Furthermore, patients had normal organ and bone marrow function measured within 4 weeks prior to administration of study treatment and agreed to use adequate contraception for the duration of the study treatment, and for 4 months thereafter. Patients who required anticonvulsant therapy had to take non-enzyme inducing antiepileptic drugs (non-EIAED); EIAED were prohibited. Patients previously on EIAED had to be switched to non-EIAED at least 2 weeks prior to start of treatment. Patients were excluded for treatment with panitumumab if they had radiotherapy within 3 months prior to the diagnosis of progression or with a dose over 65 Gy. Additional exclusion criteria included: known hypersensitivity to panitumumab, history of interstitial pneumonitis, pulmonary fibrosis, clinically significant preexisting cardiac conditions or stroke, or acute myocardial infarction within 2 months before the first dose of study treatment; ongoing toxicity of grade 2 or higher (other than alopecia) according to "Common Terminology Criteria for Adverse Events (CTCAE, version 4.03)", caused by previous treatments; concomitant treatment with any other anti-cancer therapy; presence of any other clinically significant medical condition which made it undesirable to participate in the study. Patients were considered evaluable for the primary endpoint if at least two treatment administrations of intravenous medication were completed. Non-evaluable patients were replaced and were excluded from efficacy analysis.

Study Endpoints

The primary endpoints of this study are clinical benefit (CB), defined as confirmed complete or partial response (CR/PR) or stable disease (SD) for 16 weeks or more, according to RANO (measured at least twice, at least 28 days apart), and treatment-related grade ≥ 3 adverse events (AEs) and serious adverse events (SAEs). Tumor response was reported by the local investigator in the electronic case record form (eCRF). MRI for tumor response assessment was performed at baseline and after every fourth treatment cycle (ie, every 8 weeks). If study treatment was continued after 3 response evaluations (ie, 24 weeks), response evaluations were performed at the end of every sixth treatment cycle (ie, every 12 weeks). Secondary endpoints included: objective response rate (ORR, defined as PR or CR), duration of response (DoR), PFS, and OS. Safety was measured by the frequency of treatment-related grade ≥ 3 AEs and SAEs occurring up to 30 days after the last dose of study drug. All AEs were graded according to the CTCAE v4.03. Safety within the trial is monitored by an Independent Data Monitoring Committee (IDMC) that is blinded for response rates per cohort during accrual.

Statistical Analysis

Cohorts in DRUP are monitored using a Simon-like 2-stage "admissible" monitoring plan to identify cohorts with evidence of activity.[26] If there were no patients with CB in the first 8 participants in the cohort, the cohort would be closed. Otherwise, an additional 16 patients would be included in the cohort. If 5 or more patients met the definition of CB, further investigation would be warranted. The null hypothesis and alternative hypothesis to be tested were defined as CBR of 10% versus ≥30%. This monitoring rule had 85% power to reject the null hypothesis of a CBR 10% when the true CBR is 30%, with a one-sided alpha error rate of 7.8%. Exact 95% CIs were calculated using the Clopper-Pearson method. All statistical analyses were performed using R version 4.0.3 (https://www.R-project.org). Patient

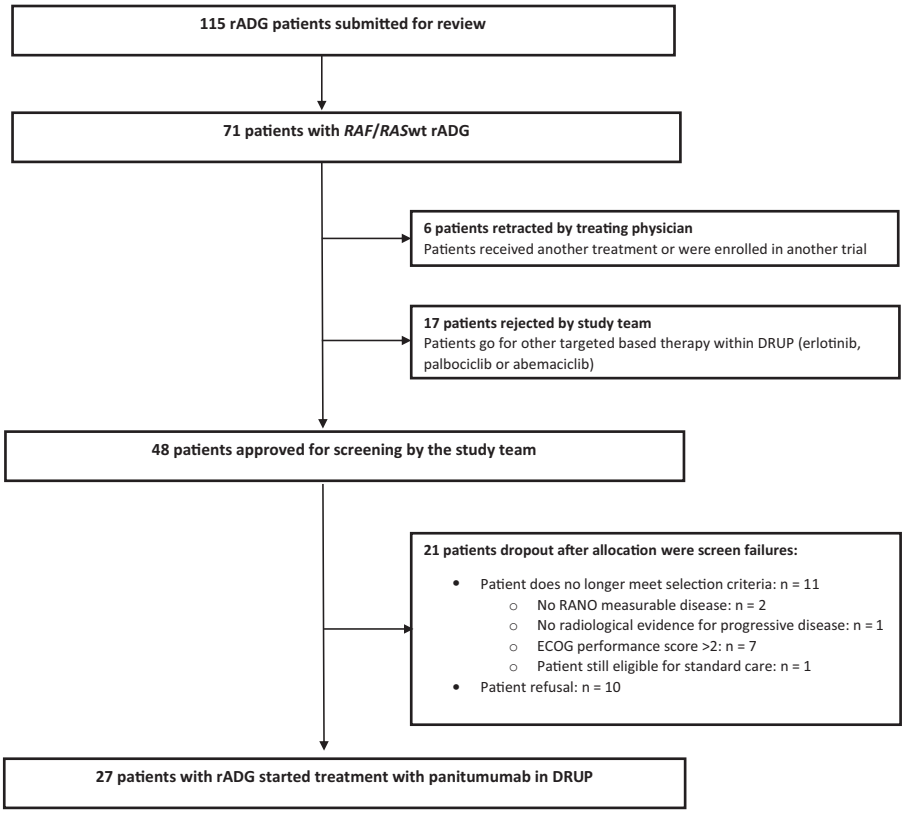

Figure 1. Flowchart of patients submitted to the study team and reasons for non-accrual. Abbreviations: rADG, recurrent adult-type diffuse gliomas; *RAF*/*RAS*wt, *RAF*/*RAS* wild-type.

characteristics, AEs, and tumor responses were summarized using descriptive statistics. A waterfall plot was used to illustrate maximum tumor shrinkage compared to baseline. Kaplan-Meier methods were used to estimate PFS (from start treatment to progression or death from any cause, whichever came first, and censoring patients alive without progression) and OS (calculated from the first day of treatment administration to the date of death from any cause, censoring patients who were alive at follow up).

Role of Funding Source

This investigator-initiated study receives funding from the Dutch Cancer Society (KWF), Stelvio for Life Foundation and receives equal funding from multiple pharmaceutical companies, including Amgen. Study medication was made available, free of charge, by the manufacturer. Amgen had no role in the design or execution of the study and no influence on the study report.

Results

Patients

Between March 2018 and January 2022, 115 patients with recurrent adult-type diffuse gliomas who had exhausted standard treatment options were submitted to the study team for evaluation of potential study participation. Seventy-one patients (65%) had a *RAF*/*RAS*wt recurrent adult-type diffuse glioma, of which 48 patients (67%) were approved by the study team to be screened for treatment with panitumumab. After screening, 27 patients (56%) were found eligible and started study treatment (Fig. 1). All 27 patients that started study treatment were included for baseline characteristics and safety analysis. Among these 27 patients, 3 patients were not evaluable for the primary endpoint and therefore excluded for the efficacy analysis. Two patients received only one complete treatment cycle due to rapid clinical deterioration and one patient had a baseline scan older than the maximum of 28 days. Baseline characteristics of the patients that were included are presented in Table 1.

Most of the evaluable patients (18/24, 75%) were included based on their primary diagnosis and had clinical and radiological progression; the other 6 (25%) patients had histologically proven recurrent glioblastoma. Pathological revision was performed to classify the tumors of the patients according to the fifth WHO Classification of Tumors of the CNS, the current leading guideline, while most patients were included based on older histologic diagnosis.[24] After revision of the pathology diagnosis, most of the included patients had a glioblastoma, *IDH* wt (n = 19, 79%). There were 2 patients with astrocytoma, *IDH* mutant grade 3 and 2 patients with astrocytoma, *IDH* mutant grade 2. These 4 patients were included based on their primary diagnosis and showed clinical and radiological evidence for dedifferentiation towards grade 4 tumors and were therefore included in this cohort. For one patient revision of pathology was not possible due to missing material.

Median time from diagnosis to start study treatment was 14 months (range 5-197 months). Patients had a median age of 54.5 years (range 35-73) and 70% (N = 19) of the patients were men. Most patients (n = 25) received the standard treatment schedule, consisting of radiotherapy with concomitant and adjuvant temozolomide,[2] two patients

Table 1. Baseline characteristics of patients enrolled in the cohort "panitumumab for patients with *RAF*/*RAS*wt recurrent glioblastoma."

Characteristics	No. of patients (%)
Median age, years (range)	54.5 (35-73)
Gender	
Male	19 (70%)
Female	8 (30%)
WHO performance status	
WHO 0	6 (22%)
WHO 1	18 (67%)
WHO 2	1 (4%)
Unknown	2 (7%)
Prior lines of systemic therapy after SoC ("temozolomide/radiotherapy")[2]	
0	16 (59%)
1	8 (30%)
2	3 (11%)
WHO classification[23]	
*IDH*wt glioblastoma	23 (85%)
IDH mutant, grade 3 astrocytoma	2 (7.5%)
IDH mutant, grade 4 astrocytoma	2 (7.5%)
Median time from first diagnosis to start study treatment, months (range)	14 (5-197)
Prognostic factors	
1.*MGMT* promoter methylation	
Yes	3 (11%)
No	14 (52%)
Unknown	10 (37%)
2.*IDH*1/2 mutation	
Yes	4 (15%)
No	23 (85%)

Abbreviations: NO, number; WHO, World Health Organization; SoC, Standard of Care

did not receive radiation therapy due to very extensive disease and were treated with only temozolomide. Eleven patients had prior second-line palliative systemic treatment of which 8 patients received bevacizumab, lomustine monotherapy, or in combination with procarbazine or hydroxyurea and 3 patients participated in a clinical trial with experimental therapy. There were no patients who received any other *EGFR* inhibitor drug prior to inclusion. There were no significant differences in baseline characteristics between patients with CB and without CB, as depicted in Supplementary Table S1.

Clinical Benefit and Survival

At data cutoff in August 2022, the median follow-up was 15.8 months (95% CI 15.2-NA months). The main reason for treatment discontinuation was progressive disease (n = 22, 81%). Three patients discontinued treatment due to symptomatic deterioration (12%) and the other 2 patients were still on study treatment. Of the 24 evaluable patients, 21% had CB (n = 5) upon treatment with panitumumab. Two patients achieved a PR and 3 patients had SD at 16 weeks. Figure 2 depicts the greatest changes in the sum of target lesions for each patient. The median PFS and OS were 1.7 months (95% CI 1.6-2.1 months) and 4.5 months (95% CI 2.9-8.6 months), respectively (Fig. 3A and 3B). The median time on treatment for the total group of patients was 1.4 months, while it was 12.7 months for the patient group with CB (95% CI 5.1-NA months).

An additional exploratory analysis in which we excluded the 4 patients with *IDH* mutated tumors showed no significant different results. All patients with *IDH* mutated tumors were patients with PD after 16 weeks of treatment. The median PFS and OS were 1.7 months (95% CI 1.6-3.5 months) and 4.5 months (95% CI 3.3-8.6 months), respectively (Supplementary Fig. S1A and S1B).

Results of Molecular Testing

As already mentioned, all patients had an *RAF*/*RAS*wt recurrent glioblastoma tumor based on molecular testing that was performed before entering DRUP. Most patients were included based on results of locally performed Next Generation Sequencing (NGS) panels (23 out of 24 evaluable patients), and one patient has had whole-genome sequencing (WGS). Figure 4 describes all detected (likely) pathogenic cancer associated other molecular alterations. In the two patients with a PR, only *TP53* or *TERT* promoter mutations were found and no other potential oncogenic driver mutations. Of the patients with SD or PD, in 13 out of 22 patients other potential oncogenic driver mutations were detected. In half of the patients (n = 12), *EGFR* alterations were found. Of these 12 patients, 6 patients had only an *EGFR* amplification, 2 patients had only an *EGFR* mutation, and 4 patients had an *EGFR* mutation and amplification. Only one patient had the common *EGFRv3* mutation, and the other patients had the following *EGFR* mutations: p.Ala289Thr (n = 1), p.Arg324Leu (n = 1), p.Arg108Lys (n = 1), p.Arg108Gly (n = 1), and p.Ala289Val (n = 1). There was no correlation between the presence of any *EGFR* alteration and clinical benefit.

Safety

Overall, panitumumab was well tolerated. No AEs > grade 3 were observed in this cohort and none of the patients discontinued study treatment due to an AE. All reported AEs are shown in Table 2. For the AEs in bold, the relation to the treatment was scored as either possible, probable, or definite.

Discussion

EGFR alterations are present in 57% of patients with glioblastomas, but treatment strategies targeting *EGFR* have thus far failed in clinical trials. The results of this cohort also demonstrate that panitumumab, an *EGFR* targeting antibody, had very limited efficacy in patients with a recurrent *RAF*/*RAS*wt glioblastoma. We observed SD in 3 out of 24 patients (12.5%) and only 2 out of 24 patients had a PR (8%). However, the median PFS of 1.7 months for the whole group is truly disappointing. Still, in the subgroup with CB, PFS was 13 months, which is favorable. All patients in the described cohort were selected based on the absence of alterations in the *KRAS*, *NRAS*, or *BRAF* gene in their tumor, as presence of these alterations lead to a well-known resistance mechanism for anti-*EGFR* targeting therapies in colorectal cancer.[27] Despite this selection of patients with *RAF*/*RAS*wt recurrent glioblastomas, our results are in line with previous reports on the activity of *EGFR* targeting therapies in patients with recurrent glioblastomas.[17-19,28,29]

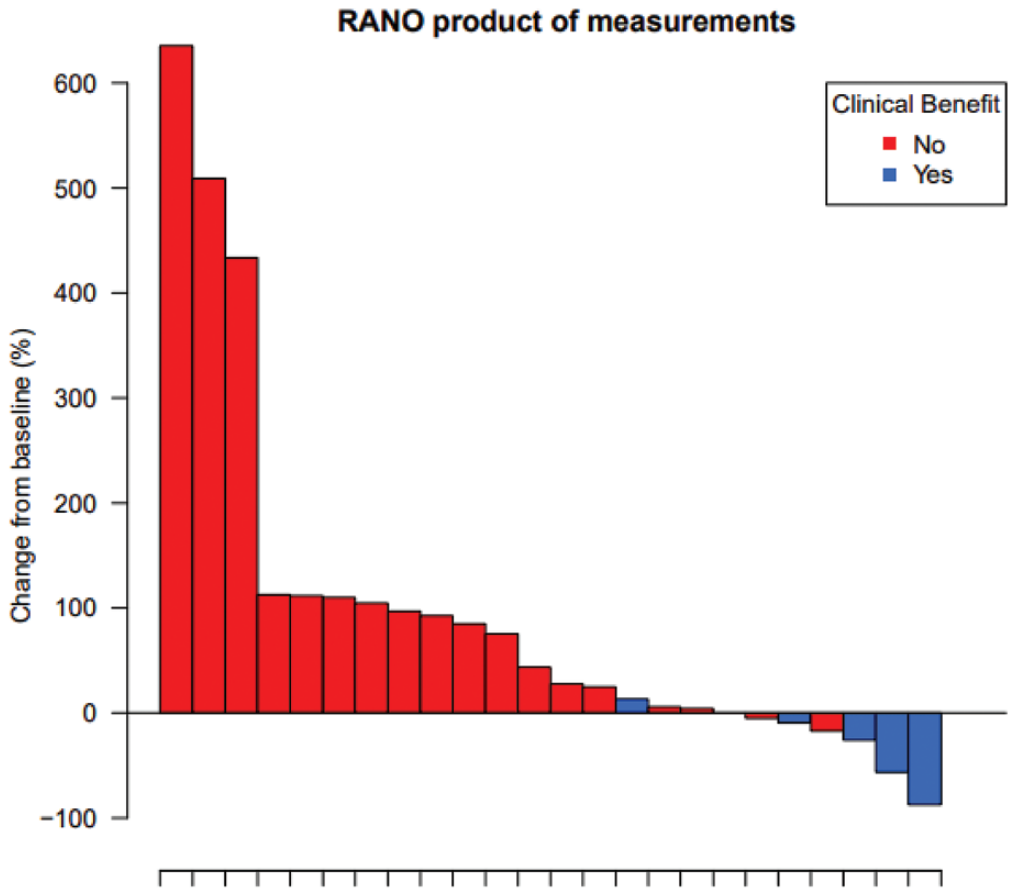

Figure 2. Waterfall plot with colors indicating the best response.

In a stratified phase II trial of cetuximab, an ORR of 5.5% and disease control rate of 29.6% were reported in 55 patients with recurrent glioblastomas. No correlation between response, survival, and *EGFR* amplification was found in this study.[18] Additionally, Westphal et al conducted a randomized, open-label phase III trial to evaluate efficacy of nimotuzumab added to standard therapy for newly diagnosed glioblastomas. Their results showed no survival benefit from adding nimotuzumab to the standard therapy, and also in this trial no correlation between *EGFR* amplification and clinical efficacy of nimotuzumab was found.[19] Aside from the *EGFR* targeting antibodies cetuximab and nimotuzumab, several first generation small-molecule tyrosine kinase inhibitors of *EGFR* were tested in clinical trials. For example, Rich et al tested gefitinib in patients with recurrent glioblastomas in a phase II trial. No objective tumor responses were seen among 53 assessable patients and only 7 patients (13%) had a 6-month event-free survival. Again, no correlation between *EGFR* expression and OS was detected.[28] In a randomized, controlled, phase II trial conducted by van den Bent et al, 110 patients with progressive recurrent glioblastomas after prior radiotherapy were randomly assigned to either erlotinib or a control arm receiving treatment with either temozolomide or carmustine. The 6 months PFS rate in the erlotinib arm was 11.4% vs 24% in the control arm.[29] Also in our trial, no correlation between *EGFR* alterations and clinical benefit was found. This is in line with previous results and known from anti-*EGFR* therapy in patients with colorectal cancer.[20]

Together, these data demonstrate that even though alterations in *EGFR* are common in patients with glioblastomas, targeting *EGFR* provides limited clinical benefit. *EGFR* is a tyrosine kinase at the upstream end of signal transduction pathway. Mutations or deregulation of downstream molecules and upregulation of redundant receptor tyrosine kinases may bypass *EGFR* inhibition.[30] For example, the presence of additional activating mutations in downstream effectors such as *PTEN*,[31] *PIK3CA*,[32] or *KRAS*[33] or co-occurrence of other amplified or mutated redundant receptor tyrosine kinases, including *MET* and *PDGFRA/B* can be responsible for treatment resistance here.[34-36]

A limitation of the current study is that we do not take into account other possible important molecular alterations related to resistance than *RAF/RAS*. Eligible patients for this trial had a treatment refractory recurrent glioblastoma with molecular testing demonstrating no mutations in either *BRAF*, *KRAS*, or *NRAS*. From all patients, an NGS panel or WGS was available before inclusion in the DRUP trial. Most of these molecular tests were performed on archived tumor material obtained by primary diagnosis, and therefore we do not have detailed information on molecular characteristics of the recurrent tumors in our cohort when study treatment was initiated. Importantly, in the 2 patients who had PR, no mutations in downstream effectors of the *EGFR* pathway were present based on the performed NGS-analyses. In 13 out of 22 patients (59%) with SD or PD, potential other oncogenic alterations were already found at primary diagnoses including mutations in downstream

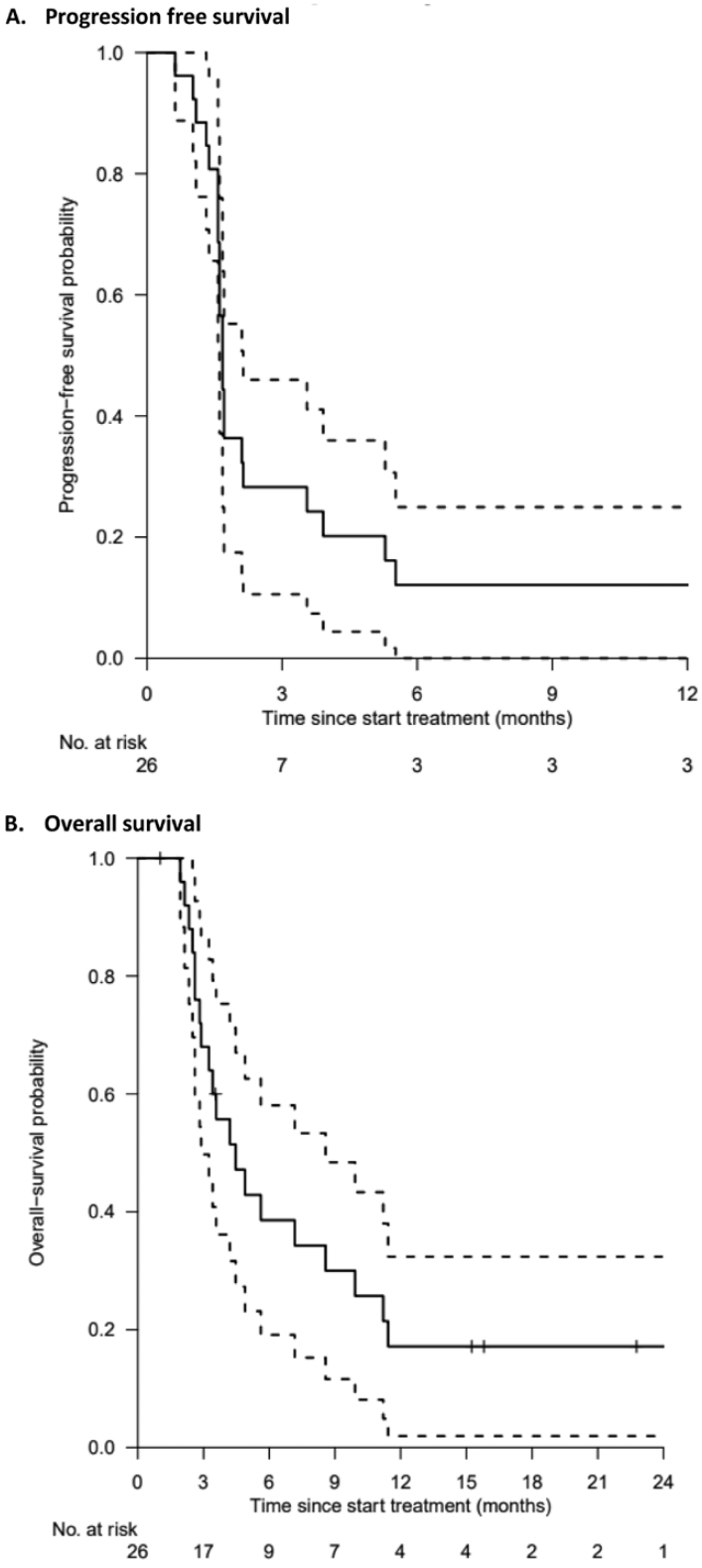

Figure 3. Progression-free survival and overall survival curves. Kaplan-Meier curve for estimated progression-free survival (**A**) and overall survival (**B**), with 95% confidence interval (dashed lines).

effectors (*PTEN* mutation: $n = 4$ and *PDGFRA* amplification: $n = 1$) and other potential driver alterations as *CDK4* amplification: $n = 2$, *NF1* mutation: $n = 1$, *CDKN2A* deletion: $n = 3$, *ATRX* mutation, $n = 1$ and *MDM2* amplification: $n = 1$. Although an overall high degree of stability in the mutational status of driver glioma genes and pathways has been shown,[37] in ~20% a mutational change at tumor recurrence is detected.[38,39] These findings could indicate that, based on additional downstream alterations, it might be possible to further select a small group of patients with recurrent glioblastomas that might benefit from anti-*EGFR* therapy. Repeated biopsies could be considered before

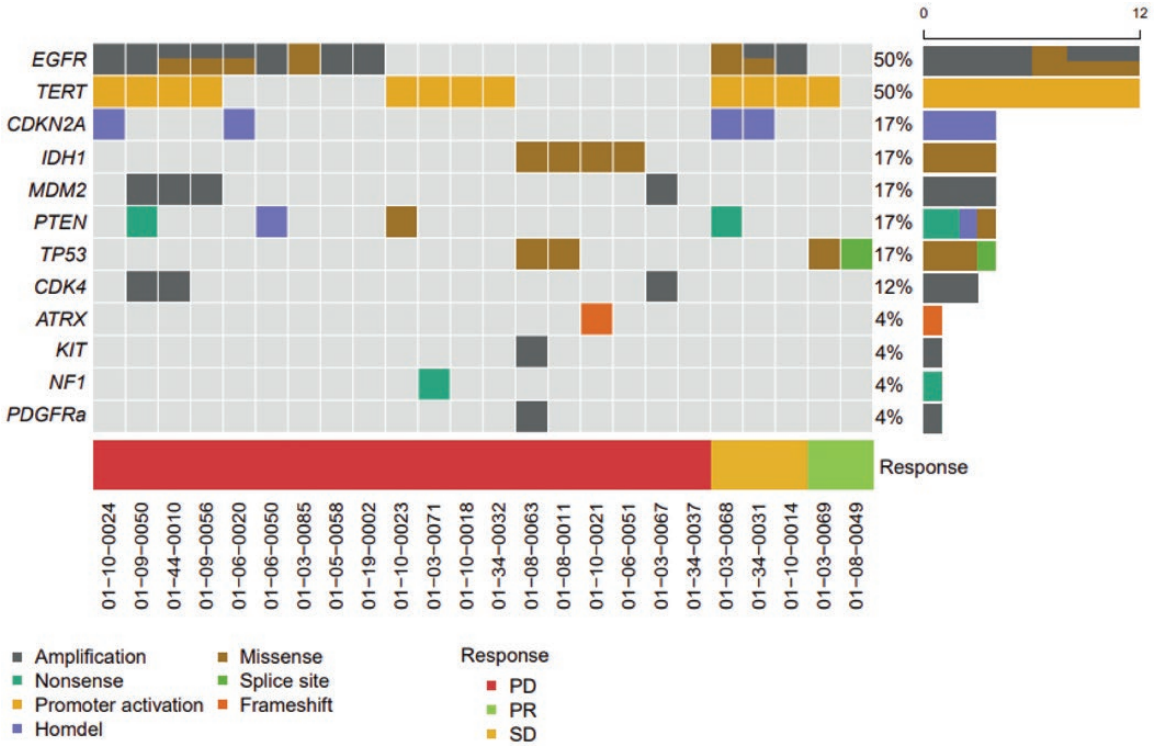

Figure 4. Oncoplot. All detected (likely) pathogenic molecular alterations by molecular testing before participating within DRUP. Abbreviations: PD, progressive disease; PR, partial response; SD, stable disease.

Table 2. Adverse events

Adverse events	Grade 1	Grade 2	Grade 3
Aphasia			1
Cerebral edema			1
Focal seizures		1	2
Hemi-anopsia			2
Lymphopenia			1
Rash	**1**		**4**
Somnolence			1
Thromboembolic event			1

For the adverse events in bold, the relation to the treatment was scored as either "possible", "probable", or "definite".

entering in targeted therapy trials to detect new driver mutations in recurrent tumors. Besides molecular alterations, the BBB may also play a role in treatment resistance in glioblastomas. Whether the BBB plays a role as a potential resistance mechanism for panitumumab treatment is unknown. There is some preclinical and clinical evidence suggesting that the BBB prohibits achievement of therapeutic drug concentrations of tyrosine kinase inhibitors (TKIs) in brain tumors.[40] This kind of evidence is currently not available for monoclonal antibodies in recurrent glioblastomas from which it is expected that the BBB is disturbed; however, it is known that only 0.1%-0.2% of administered therapeutic antibodies cross the BBB when intact.[41] This seems logical as monoclonal antibodies are larger molecules compared to TKIs. Previously, it has been suggested that future treatment benefits can be achieved through intra-arterial delivery of medicines into the brain across an osmotically opened BBB, instead of using the intravenous route.[42] This has been demonstrated for bevacizumab, also a monoclonal antibody. Therefore, it might be that intra-arterial delivery of panitumumab could be more effective than i.v. panitumumab treatment. However, VEGF as the target of bevacizumab is mainly present in the vascularization in contrast to the target of panitumumab. Other previous research showed that intra-tumoral concentration and efficacy of antibody-drug conjugates in patients with glioblastoma inversely correlated with tumor size.[43] Aside the fact that we selected patients only based on RAF/RASwt and did not take other molecular alterations, nor the glioblastomas specific intratumor heterogeneity into account, other important limitations of this study were the absence of both randomization and a control group.

Revision of pathology diagnoses revealed 4 patients with *IDH* mutant astrocytoma. These tumors are known to behave different compared to *IDH*wt glioblastomas. However, a sub-analysis in which we excluded these patients did not significantly change our results. Although we expected that patients with *IDH* mutated tumors might have had a more favorable outcome based on their tumor characteristics, they all had progressive disease after 8 weeks of treatment.

Conclusion

In patients with a recurrent glioblastoma, therapy selection based on *RAF*/*RAS*wt genotyping for panitumumab provided insufficient CB to be further explored as a treatment strategy despite the fact that it was well tolerated. We believe that

future research should focus on the delivery of drugs through the BBB and unravelling resistance mechanisms by broader genomic evaluation.

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Conflict of Interest

The authors declare the following financial interests / personal relationships which may be considered as potential competeting interests: Derk-Jan A. de Groot received funding for an investigator-driven phase II study from Hofmann la Roche and a PUSH grant for a PhD student from Siemens. All outside the submitted work and all money had been received by the UMCG. Emile E. Voest reported research funding for DRUP from Amgen. The other authors declare no conflicts of interests.

Author Contributions

Conception/design: I.A.C.S., B.S.G., L.J.Z., G.F.d.W., V.v.d.N., H.A.J.G., E.E.V., H.M.W.V. Provision of study material or patients: I.A.C.S., B.S.G., L.J.Z., G.F.d.W., P.R., W.W.J.d.L., A.M.L.J., L.V.B., F.d.V., J.A.d.G., J.W.B.d.G., A.H., J.B., H.A.J.G., E.E.V., H.M.W.V. Collection and/or assembly of data: I.A.C.S., B.S.G., L.J.Z., G.F.d.W., B.K. Data analysis and interpretation: I.A.C.S., V.v.d.N., B.K., H.M.W.V. Manuscript writing: I.A.C.S., H.M.W.V. Final approval of manuscript: All authors.

Data Availability

All data described in this study are freely available for academic use and can be obtained through a request to the corresponding author by email.

Supplementary Material

Supplementary material is available at *The Oncologist* online.

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