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ORIGINAL ARTICLE

Safety, tolerability, and pharmacokinetics of anti-EGFRvIII antibody–drug conjugate AMG 595 in patients with recurrent malignant glioma expressing EGFRvIII

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Abstract

Purpose Epidermal growth factor receptor variant III (EGFRvIII) is expressed in a significant percentage of primary and recurrent glioblastoma (GBM), a common malignant primary brain tumor in adults. AMG 595 is an antibody–drug conjugate comprising a fully human, anti-EGFRvIII monoclonal antibody linked to DM1. The study goals were to assess safety, tolerability, and pharmacokinetics of AMG 595 in GBM.

Methods In this phase 1, first-in-human, open-label, sequential-dose, exploration study, adults with recurrent GBM received AMG 595 once every 3 weeks (Q3W) according to incremental dosing cohorts (0.5–3.0 mg/kg). Primary endpoints were to assess safety, the incidence of dose-limiting toxicities (DLTs), objective response (per Macdonald criteria), evaluate pharmacokinetics, and estimate the maximum tolerated dose (MTD).

Results Of 382 patients screened, 32 were enrolled and received ≥ 1 dose of AMG 595. Ten patients experienced 18 DLTs (all grade 4 thrombocytopenia), and the MTD was 2.0 mg/kg. Twenty-eight patients (88%) experienced ≥ 1 treatment-related adverse event (AE); the most common AEs were thrombocytopenia (50%) and fatigue (25%). Grade ≥ 3 treatment-related AEs occurred in 17 patients (53%); 11 (34%) had serious treatment-emergent AEs, and none were considered treatment related. Pharmacokinetic profiles indicated low levels of circulating unconjugated antibody and cytotoxin, dose-proportional increases in plasma exposures for the conjugated antibody over the studied range, and less than twofold accumulation following multiple Q3W dosing. Two patients (6%) had partial responses; 15 (47%) had stable disease.

Conclusions AMG 595 exhibited favorable pharmacokinetics and is a unique therapy with possible benefit for some patients with EGFRvIII-mutated GBM with limited therapeutic options.

Keywords AMG 595 · Antibody–drug conjugate · DM1 · EGFRvIII · Glioblastoma

Introduction

An estimated 26,170 cases of primary malignant brain and other central nervous system (CNS) tumors are expected to be diagnosed in the United States in 2019 [1]. Glioblastoma (GBM) is the highest grade glioma and is the most common

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malignant primary brain tumor in adults. Despite available treatment options, most patients with recurrent disease survive < 1 year [2]. Additionally, GBM and other malignant gliomas, such as anaplastic astrocytoma (AA), can exhibit pronounced genetic heterogeneity and instability [3], which may confer resistance and, consequently, poor chemosensitivity [4]; therefore, treatment remains a significant unmet need.

The epidermal growth factor receptor (EGFR) gene has been reported to be highly amplified in a substantial proportion of primary and recurrent GBM [4], and approximately 50% of such tumors have a mutation in which exons 2–7 are deleted, resulting in a splice variant between exons 1 and 8, termed "EGFR variant III" (EGFRvIII) [3, 5]. Similar to native EGFR, EGFRvIII is a membrane-bound receptor; however, the deletion results in a protein that lacks the extracellular ligand-binding domain and has constitutive tyrosine kinase activity that promotes malignant growth [3]. At present, there is no evidence of EGFRvIII expression in wild-type human tissues; thus, EGFRvIII serves as a unique tumor-specific antigen and is a candidate for targeted therapy.

AMG 595 is an antibody–drug conjugate comprising a fully human, anti-EGFRvIII monoclonal antibody linked to the maytansinoid DM1, a semisynthetic derivative of maytansine. AMG 595 binds to EGFRvIII but not native EGFR; after binding, the AMG 595–EGFRvIII complex is internalized via the lysosomal pathway, leading to the release of DM1 and mitotic arrest [6]. In preclinical studies, the optimal drug–antibody ratio (i.e., anti-EGFRvIII:DM1) for AMG 595 was determined to be approximately 3.5 [7]. Additionally, AMG 595 showed potent in vitro and in vivo cytotoxic activity against GBM cells expressing EGFRvIII [7]. AMG 595 significantly delayed tumor growth and initiated tumor regression compared with controls in tumor xenografts [7].

This was a phase 1, first-in-human study of AMG 595 in patients with recurrent GBM. The primary objectives were to evaluate the safety, tolerability, and pharmacokinetics of AMG 595, determine the maximum tolerated dose (MTD), and assess whether the objective response rate per local read at the MTD was > 20%.

Materials and methods

Patients

Adult patients (aged ≥ 18 years) with GBM or AA at the time of the first or second recurrence following initial therapy (e.g., surgery with or without adjuvant radiation or chemotherapy) who met the following criteria were enrolled: Karnofsky performance score ≥ 70%; ≥ 1 site of bidimensionally measurable disease; life expectancy ≥ 3 months; and adequate hematologic (absolute neutrophil count $\geq 1.5 \times 10^9$/L; platelet count $\geq 100 \times 10^9$/L; hemoglobin > 9 g/dL), renal, and hepatic function. Patients with a history of CNS bleeding, evidence of acute intracranial/intratumoral hemorrhage, peripheral sensory neuropathy of grade > 2, or who had experienced a myocardial infarction within 6 months of enrollment were excluded. *IDH1* mutation status was not collected for enrolled patients. Patients were required to provide archived tumor tissue (taken at either initial occurrence or after tumor recurrence) at enrollment, as enrollment was restricted to patients who showed evidence of EGFRvIII expression in tumor tissue as assessed by a prototype EGFRvIII immunohistochemistry (IHC) assay (Dako North America, Inc., an Agilent Technologies Company, Carpinteria, CA) [8] on sections containing a minimum of 100 evaluable tumor cells. The variable region of a novel anti-EGFRvIII antibody developed using XenoMouse technology was combined with the murine immunoglobulin G constant region to create an appropriate IHC reagent for human tissue for this kit [8]. The antibody used for the IHC assay and the antibody component of AMG 595 had identical specificity and affinity for EGFRvIII; thus, the combination resulted in an antibody that had no cross-reactivity with other targets or with wild-type EGFR. Patients were considered to have EGFRvIII-positive tumors if their specimen contained ≥ 1% of tumor cells staining at any intensity (in cytoplasm or on the membrane) or if < 1% of cells stained positive in the IHC section and there was a pattern of positive-staining cells on the growing margin of the tumor impinging into normal brain tissue. The intracellular staining for EGFRvIII was not intended to indicate the location of the target for the antibody–drug conjugate, but rather any cells expressing EGFRvIII in the cytoplasm were classified as positive; it was assumed that intracellular expression would indicate that some mutant receptors were likely expressed on the externally facing cell membrane.

Ethical approval

The study protocol was approved by an institutional review board or independent ethics committee at each study site and was conducted in accordance with International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) regulations/guidelines. All patients provided written informed consent. This trial is registered with ClinicalTrials.gov, identifier NCT01475006.

Study design and treatment

This was a first-in-human, open-label, sequential-dose, exploration study of AMG 595 comprising dose-escalation (Part 1) and dose-expansion (Part 2) phases conducted

between February 28, 2012 (first patient enrolled), and April 5, 2016 (last patient completed follow-up), at four study centers in the United States and Australia (Supplementary Figure S1). The primary endpoints were to assess safety and the incidence of dose-limiting toxicities (DLTs), to evaluate pharmacokinetics, to estimate the MTD (defined as the maximum dose at which the probability of a DLT is ≤ 25%), and to assess objective response (complete or partial response assessed by Macdonald criteria) [9]. Secondary endpoints included duration of response, time to response (TTR), and incidence of anti-AMG 595 antibody formation.

The screening of most patients in the study was undertaken on tumor tissue obtained during initial debulking surgery following initial diagnosis. Patients were eligible for enrollment if their tumor was determined to be EGFRvIII positive as described above. However, most patients were screened and enrolled in the study at the time of first or second recurrence and did not have surgical resection or biopsy at that time. Therefore, the determination of EGFRvIII positive was based on the initial occurrence and not the recurrence of the tumor. Patients received intravenous AMG 595 once every 3 weeks (Q3W) as a 60-min infusion on study day 1 of each 3-week cycle. Dosing continued until disease progression, intolerance, or consent withdrawal. In Part 1, three to five patients were enrolled sequentially into cohorts beginning at the lowest dose. Dosing was then escalated in prespecified increments (0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/kg) using a practical continual reassessment method (CRM) [10]; intermediate doses (multiples of 0.5 mg/kg) could be used as required.

A DLT was defined as any occurrence of febrile neutropenia within the first 28 days of each dose: grade ≥ 3 neutropenia associated with infection; grade 4 neutropenia lasting > 7 days; grade 3 thrombocytopenia lasting > 7 days or with significant hemorrhage (e.g., gastrointestinal bleeding, intracranial hemorrhage); grade 4 thrombocytopenia; grade ≥ 3 anemia not attributable to other causes; grade ≥ 3 nausea, vomiting, or diarrhea despite optimal medical support; grade 3 fatigue lasting > 7 days; grade 4 fatigue; or any other grade ≥ 3 adverse event (AE; except alopecia). Any patient who met the requirements for a Hy's law case of drug-induced injury (defined as alanine aminotransferase or aspartate aminotransferase values ≥ 3 × upper limit of normal [ULN], a total bilirubin level > 2 × ULN, and lack of alternative clinical explanation for these findings) was also considered to have a DLT. Patients who required longer than 4 weeks to recover from treatment-related grade 3 or 4 nonhematologic toxicities were permanently discontinued from the study.

If no DLTs occurred within the first 28 days in the initial cohort, then dose escalation to the next highest nominal dose level occurred. This process continued until all nominal doses were studied or a DLT was observed. When the first DLT was observed, the dose for the next cohort was based on prediction of the MTD using a model for the dose–toxicity curve. The dose closest to the predicted 25% DLT probability was selected out of the available nominal and intermediate doses; the maximum dose increase at any point was ≤ 2 times the current dose. Dose exploration was stopped if ≥ 2 DLTs were observed within the first 28 days in the first three patients of Cohort I, when the probability of a DLT for the dose closest to the predicted MTD was estimated to a suitable precision (i.e., upper 95% confidence interval [CI]/lower CI ≤ 2), or if all nominal doses were tested and no DLTs had been observed within the first 28 days. If none were observed, the maximum administered dose was used for Part 2. Part 2 began upon completion of Part 1; patients in Part 2 were treated at the MTD determined during Part 1.

For Part 1, a dose-level review meeting was held to review data, monitor safety, and make dose and schedule change decisions; for Part 2, dose-level review meetings were planned to be held after 10, 20, and 30 patients had been enrolled at the MTD and completed 28 days on study. Individual patient dosing was interrupted for any patient who experienced a DLT within or outside of the first 28 days of treatment in either Part 1 or 2. Dosing was held for patients who experienced a grade ≥ 3 AE or serious AE, unrelated to AMG 595, until the toxicity had resolved to a grade ≥ 1; patients taking > 3 weeks to recover from toxicities of grade ≥ 3 were withdrawn from the study.

Assessments

Adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA) and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. After the first two doses of AMG 595 and completion of the 28-day window for DLTs, patients underwent radiologic assessment using MRI during week 5; subsequent tumor evaluations by MRI occurred at week 9 and every 8 weeks thereafter. Tumor response was assessed per Macdonald criteria [9]. Patients with an objective response (defined as a complete or partial response) received a second MRI scan for confirmation within 4 weeks after the criteria for response were met.

Pharmacokinetic analyses

Both the large- and small-molecule components of AMG 595 were quantified in patients with GBM using validated bioanalytical methods. AMG 595-conjugated antibody (i.e., anti-EGFRvIII antibody conjugated to at least one DM1 molecule) and the total anti-EGFRvIII antibody (i.e., sum of unconjugated anti-EGFRvIII antibody and AMG 595-conjugated antibody) were quantified using a validated electrochemiluminescence immunoassay with a lower limit of

quantification (LLOQ) of 20 ng/mL. Total unconjugated DM1 was quantified using the microsampling dried blood spot sample collection with liquid extraction sample preparation procedure, followed by liquid chromatography–tandem mass spectrometry (LC–MS/MS) detection with an LLOQ of 2.5 ng/mL. All samples analyzed using this method were below the LLOQ. Subsequently, to improve the sensitivity by tenfold (LLOQ, 0.25 ng/mL), a more sensitive bioanalytical method was developed to measure DM1 in plasma and involved a tris(2-carboxyethyl)phosphine reduction process for sample preparation, followed by *N*-ethylmaleimide derivatization and LC–MS/MS detection. DM1 concentration data were collected for the starting dose group (0.5 mg/kg, Cohort I) and the dose-expansion group at the MTD (2 mg/kg, dose-expansion cohort). Noncompartmental methods were used to assess pharmacokinetic parameters, including maximum observed concentration (C_{max}), area under the concentration–time curve over the 3-week dosing interval ($AUC_{0–3week}$) of AMG 595-conjugated antibody, total anti-EGFRvIII antibody, and total DM1.

Statistical analysis

The sample size in Part 1 was determined empirically and was consistent with this type of first-in-human study using a CRM [10]. Analysis of DLTs was conducted on the dose-exploration analysis set, which consisted of all DLT-evaluable patients [defined as all patients who completed the DLT interval (days 1–28) and received ≥ 2 planned doses of AMG 595 or experienced a DLT]. Analyses of all other endpoints were conducted using the safety analysis set (defined as all patients enrolled and who received ≥ 1 dose of AMG 595). Descriptive statistics were used to summarize select demographics and safety.

Results

Patients

Between February 28, 2012, and November 18, 2014, 328 patients were screened and 32 patients were enrolled in the study and received ≥ 1 dose of AMG 595; the majority of disease-related screen failures occurred because patients were determined to have EGFRvIII-negative tumors (Supplementary Figure S2). Demographic and baseline characteristics are listed in Table 1. Overall, 75% of patients were men and 97% were white. The median age was 57 years (range 39–73 years), and all but 2 of 32 patients had grade IV disease at enrollment. At data cutoff, all patients had discontinued treatment, with most discontinuations due to disease progression (81.3%; Supplementary Figure S2). The median number of doses of AMG 595 was 2 (range 1–32), the median cumulative dose was 487.0 mg (range 62.0–4320.0 mg), and the median average dose delivered was 139.5 mg (range 31.0–310.0 mg).

Table 1 Patient demographic and baseline clinical characteristics

Parameters	All patients ($N = 32$)
Male, *n* (%)	24 (75)
Race, *n* (%)	
White	31 (97)
Asian	1 (3)
Ethnicity, *n* (%)	
Hispanic/Latino	1 (3)
Not Hispanic/Latino	31 (97)
Age, median (range), year	57 (39–73)
Primary tumor type, *n* (%)	
Glioblastoma multiforme	30 (94)
Anaplastic astrocytoma	2 (6)
Disease stage at time of enrollment, *n* (%)	
Grade III	2 (6)
Grade IV	30 (94)
Time since diagnosis, median (range), month	12.2 (4.9–34.3)

Safety analysis set: all enrolled patients who received ≥ 1 dose of AMG 595

Dose-limiting toxicities and maximum tolerated dose

Of the 31 patients included in the dose-exploration analysis set, 10 experienced 18 DLTs (2.0 mg/kg, $n = 3$; 2.5 mg/kg, $n = 5$; 3.0 mg/kg, $n = 2$); all were grade 4 thrombocytopenia (Supplementary Table S1). The MTD of AMG 595 was established per protocol. In brief, during the dose escalation when the 2.0 mg/kg dose (Cohort III) was initially investigated, transient thrombocytopenia that did not meet DLT criteria was noted in 67% ($n = 2/3$) of patients. Accordingly, the dose was escalated to 3.0 mg/kg (Cohort IV) per protocol. At that dose, DLTs of thrombocytopenia were noted in 50% ($n = 2/4$) of patients, and 3.0 mg/kg was determined to be not tolerated. Consequently, the dose was reduced per protocol to 2.5 mg/kg (Cohort V); however, at that dose 83% ($n = 5/6$) of patients experienced thrombocytopenia that met the DLT criteria. It was decided to reduce the dose back to 2.0 mg/kg and add three more patients at that dose (Cohort VI), 67% ($n = 2/3$) of whom experienced thrombocytopenia that met the DLT criteria. Further evaluation of this dose in additional patients established the MTD of AMG 595 to be 2.0 mg/kg. For the eight patients treated at 2.0 mg/kg who experienced an event of thrombocytopenia of any grade ($n = 32$ events), the median duration of the episode was 4 days (range 1–71 days), and the median for lowest platelet counts observed was 143 10^9/L (range 130–156 10^9/L).

Safety

All 32 patients had ≥ 1 treatment-emergent AE, and 28 (88%) experienced ≥ 1 treatment-related AE (Table 2). The most common treatment-related AEs were thrombocytopenia (50%) and fatigue (25%). Grade ≥ 3 treatment-related AEs occurred in 17 patients (53%); the most common were thrombocytopenia (44%) and fatigue (6%; Table 2). Eleven patients (34%) had serious treatment-emergent AEs; however, none were considered treatment related. Two patients (6%) had fatal AEs; both AEs were from GBM disease progression and were not considered treatment related by investigators. Three patients (9%) had AEs that led to discontinuation of AMG 595 (thrombocytopenia, $n = 2$; fatigue, $n = 1$). All patients were negative for anti-AMG 595 binding and neutralizing antibodies at any time during the study.

Pharmacokinetics

Following intravenous administration of 0.5–3.0 mg/kg AMG 595 Q3W in patients with recurrent EGFRvIII-expressing GBM, similar pharmacokinetic profiles were observed over the 3-week dosing interval for AMG 595-conjugated antibody (i.e., anti-EGFRvIII antibody with at least one DM1 molecule conjugated to the antibody) and total anti-EGFRvIII antibody (i.e., sum of unconjugated anti-EGFRvIII antibody and AMG 595-conjugated antibody), indicating low levels of circulating unconjugated antibody. In addition, low plasma levels were observed for DM1 (total unconjugated DM1) (mean $C_{max} < 2$ ng/mL) (Fig. 1).

After intravenous administration of 0.5–3.0 mg/kg AMG 595 Q3W, plasma exposures for AMG 595-conjugated antibody and total anti-EGFRvIII antibody increased in a dose-proportional manner over the entire AMG 595 dose range (0.5–3.0 mg/kg). Following the first dose, AMG 595-conjugated antibody and total anti-EGFRvIII antibody C_{max} and $AUC_{0–3week}$ increased approximately sixfold and sevenfold, respectively, for the sixfold increase in dose (0.5–3.0 mg/kg). Based on a comparison of values for mean C_{max} and $AUC_{0–3week}$ across the dose range tested, as well as linear regression analysis of dose-normalized log-transformed C_{max} and $AUC_{0–3week}$ values, AMG 595-conjugated antibody and total anti-EGFRvIII antibody exposure increased dose proportionally from 0.5 to 3.0 mg/kg. Minimal (less than twofold) accumulation was observed following intravenous administration of AMG 595 Q3W, with mean accumulation ratios ranging from 1.33 to 1.53 for AMG 595-conjugated antibody and from 1.21 to 1.67 for total anti-EGFRvIII antibody. No patients tested positive for anti-AMG 595 antibodies at any post-dose time points; therefore, the impact of antibodies on AMG 595-conjugated antibody, total EGFRvIII antibody, and DM1 could not be assessed. As shown

Table 2 Adverse events

Adverse event	All patients ($N = 32$)
All treatment-emergent AEs	32 (100)
Grade ≥ 3 AEs	25 (78)
Serious AEs	11 (34)
Fatal AEs	2 (6)
Treatment-related AEs	28 (88)
Grade ≥ 3 AEs	17 (53)
Serious AEs	0
Fatal AEs	0
Treatment-emergent AEs with ≥ 10% incidence	
Thrombocytopenia	16 (50)
Headache	12 (38)
Fatigue	11 (34)
Nausea	11 (34)
Hemiparesis	8 (25)
ALT increased	6 (19)
Constipation	6 (19)
Hypophosphatemia	6 (19)
Upper respiratory tract infection	6 (19)
Decreased appetite	5 (16)
AST increased	4 (13)
Oral candidiasis	4 (13)
Rash	4 (13)
Seizure	4 (13)
Treatment-related AEs with ≥ 10% incidence	
Thrombocytopenia	16 (50)
Fatigue	8 (25)
ALT increased	6 (19)
Nausea	6 (19)
AST increased	4 (13)
Hypophosphatemia	4 (13)
All treatment-related grade 3 AEs	
Thrombocytopenia	4 (13)
Fatigue	2 (6)
ALT increased	1 (3)
AST increased	1 (3)
Somnolence	1 (3)
Neutropenia	1 (3)
Decreased platelet count	1 (3)
Purpura	1 (3)
All treatment-related grade 4 AEs	
Thrombocytopenia	10 (31)

All values are presented as *n* (%)

Safety analysis set: all enrolled patients who received ≥ 1 dose of AMG 595

AE adverse event, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase

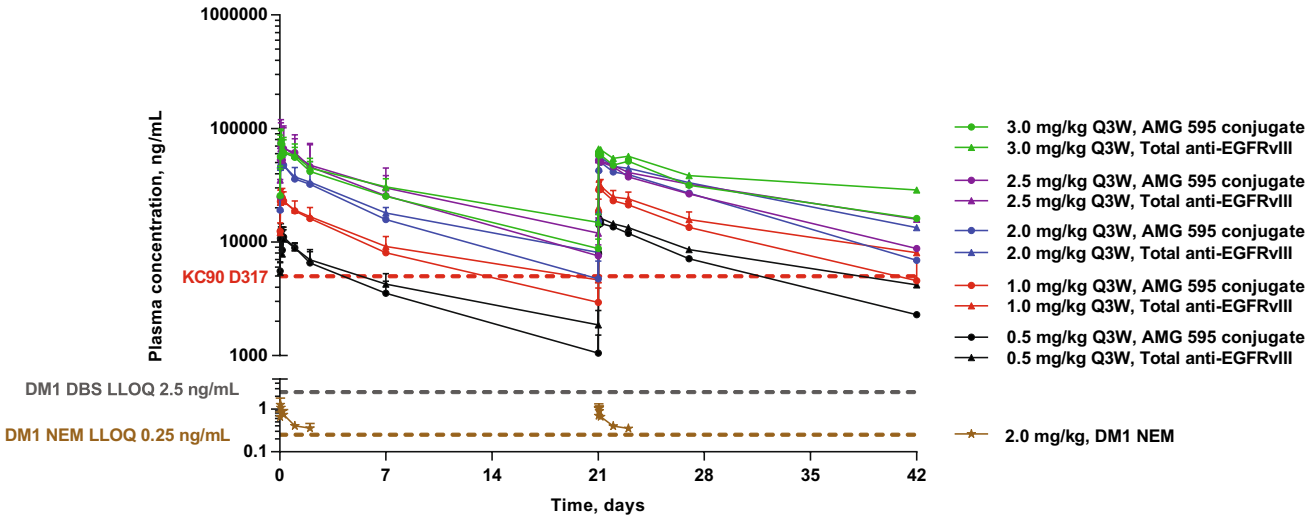

Fig. 1 Mean plasma concentration versus time after treatment with AMG 595 in patients with recurrent glioblastoma multiforme. The red dashed line marks the 90% tumor cell killing concentration from the EGFRvIII-positive D317 subcutaneous xenograft model [7] in mice. EGFRvIII, epidermal growth factor receptor variant III; Q3W, every 3 weeks; DM1 DBS LLOQ 2.5 ng/mL, bioanalytical method to determine the lower limit of quantification; DM1 NEM LLOQ 0.25 ng/mL, improved sensitivity method to determine the lower limit of quantification; KC90 D317, projected efficacious AMG 595-conjugated antibody exposures (90% tumor cell killing concentration, 4990 ng/mL) determined from translational pharmacokinetic/pharmacodynamic modeling

Table 3 Best tumor response according to Macdonald criteria per local read

Tumor response	All patients ($N=32$)
Best overall response assessment	
Complete response	0
Partial response	2 (6)
Stable disease	15 (47)
Progressive disease	15 (47)

Safety analysis set: all enrolled patients who received ≥ 1 dose of AMG 595

All values are presented as *n* (%)

by the dashed red line in the concentration versus time plot (Fig. 1), in patients who received AMG 595 doses of ≥ 1 mg/kg, trough plasma concentrations remained above the 90% tumor cell killing concentration (4990 ng/mL) determined from translational pharmacokinetic/pharmacodynamic modeling [11] of tumor regression in an EGFRvIII-positive D317 subcutaneous xenograft model [7] in mice for ≥ 42 days.

Efficacy

Tumor response per Macdonald criteria by local read for patients in each cohort is listed in Table 3. Although no evidence of dose response was noted for AMG 595, most patients received a dose in Part I that was well below the MTD (2.0 mg/kg). Overall, two patients (6%) had a partial response (Table 3, Fig. 2), and 15 (47%) had stable disease (median duration of stable disease, 127 days; range, 29–421 days; Table 3); no complete responses by local read were obtained. For the two patients with a partial response, duration of response was longer than expected for patients with recurrent GBM (393 and 576 days, respectively), and TTR was 227 and 118 days at data cutoff.

Discussion

This phase 1, first-in-human study evaluated the safety/tolerability, pharmacokinetics, and objective response rate of AMG 595 in patients with recurrent GBM. Eighteen DLTs were observed in ten patients; all were grade 4 thrombocytopenia. Low levels of circulating unconjugated antibody and a dose-proportional increase in plasma exposure were observed. Two patients (6%) had a partial response and 15 (47%) had stable disease; no complete responses occurred. The MTD for AMG 595 in this study was determined to be 2.0 mg/kg, which is comparable with the MTD (1.5 mg/kg) and recommended phase 2 dose (1.25 mg/kg) of depatuxizumab mafodotin (formerly ABT-414), an antibody–drug conjugate designed to target overexpressed EGFR in patients with glioblastoma [12], and the MTD and recommended phase 2 dose (3.6 mg/kg) of ado-trastuzumab–emtansine (trastuzumab–DM1), an antibody–drug conjugate for human epidermal growth factor receptor 2 (HER2)-positive breast

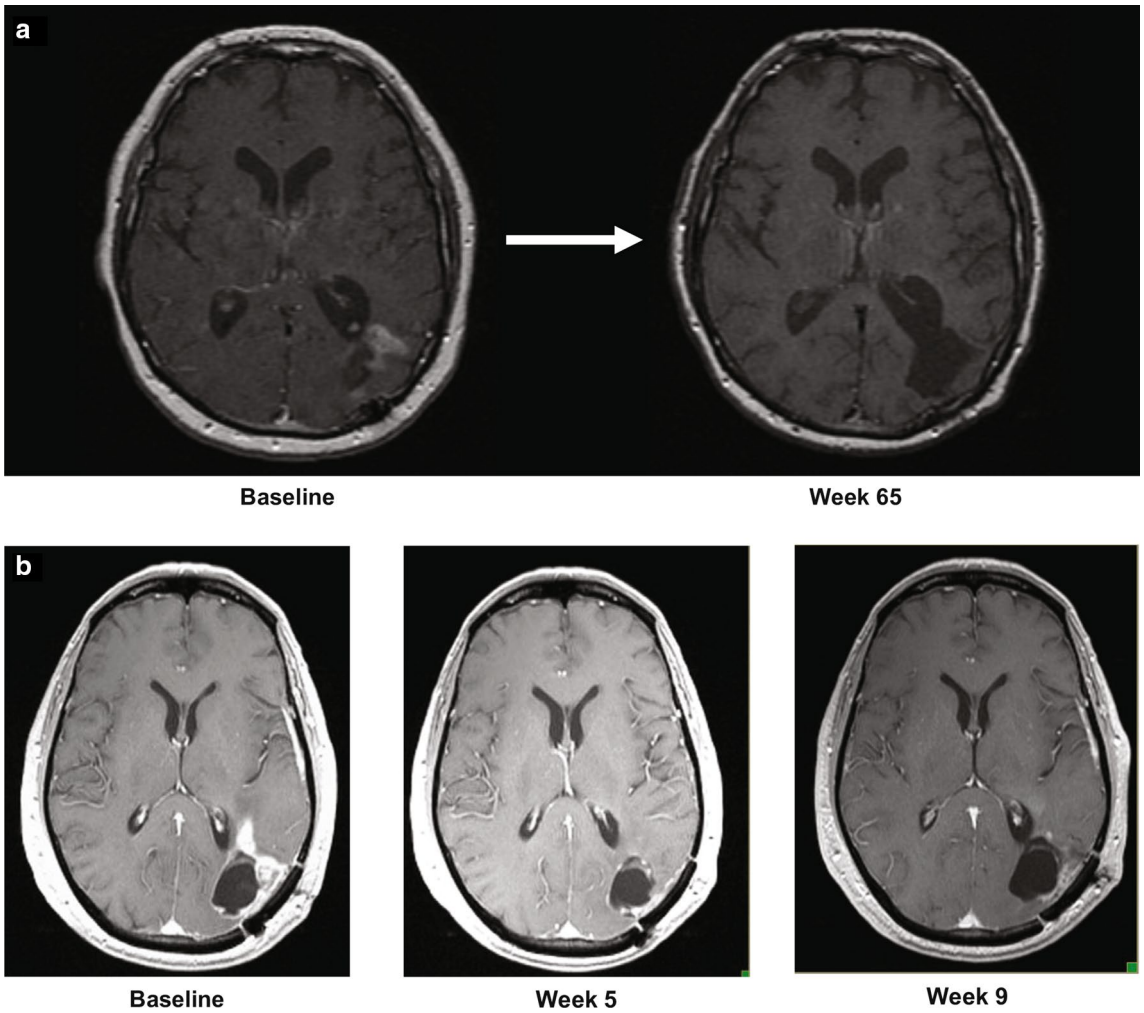

Fig. 2 MRI images from patients who had a partial response. **a** Patient had undergone previous surgery, chemotherapy, and radiation before enrollment. After 67 weeks on study, the patient experienced a near-complete response and now is asymptomatic, does not require treatment with steroids, and has returned to work. **b** Patient showed neurological improvement compared with pretreatment observation; after treatment no neurological signs were observed, the patient was off steroids, and had returned to work. The patient was on study for approximately 20 weeks before progression

cancer [13] that uses the same linker and antibody loading scheme as AMG 595 [7, 14].

Overall, treatment with AMG 595 had an acceptable safety profile in patients with recurrent GBM or AA; the most common treatment-related AEs were thrombocytopenia and fatigue, which aligned with our expectations based on the AEs observed in clinical trials with trastuzumab–DM1. In a phase 2a study of trastuzumab–DM1, paclitaxel, and pertuzumab in patients with HER2-positive breast cancer, 80% of patients experienced fatigue and 16% experienced thrombocytopenia [13]. Of note, further studies evaluating trastuzumab–DM1 have demonstrated occurrence of thrombocytopenia early in the course of treatment, with the incidence of thrombocytopenia declining with continued dosing [15]. Moreover, study results suggest that a decrease in platelet number did not detrimentally affect platelet function [15].

The results observed in this study indicate that the pharmacokinetic profile of AMG 595 in patients with recurrent GBM is consistent with the design of this antibody–drug conjugate and with previous data from studies evaluating trastuzumab–DM1 [14]. Specifically, similar profiles were observed between AMG 595-conjugated and total anti-EGFRvIII antibodies indicating low levels of circulating unconjugated antibody, which could have otherwise compromised efficacy by competing with AMG 595-conjugated antibody. Additionally, the low levels of unconjugated total DM1 present in samples collected indicated minimal exposure to the unbound antitubulin agent. A linear dose–exposure relationship was observed for the AMG 595-conjugated

antibody, indicating saturation of target at the studied doses and lack of any observable effect of target-mediated drug disposition on dose–exposure relationship. The linear nature of exposure versus dose is consistent with other antibody–drug conjugate treatments in GBM [12], and the lack of antidrug antibodies indicates that pharmacokinetic results were not confounded by their presence.

In addition, AMG 595 doses ≥ 1 mg/kg Q3W provided patients with plasma trough coverage for projected efficacious AMG 595-conjugated antibody exposures (90% tumor cell killing concentration, 4990 ng/mL) determined from translational pharmacokinetic/pharmacodynamic modeling [11] of tumor regression in EGFRvIII-positive D317 subcutaneous and intracranial orthotopic xenograft models [7] in mice. Hence, AMG 595 doses ≥ 1 mg/kg Q3W were expected to be potentially efficacious, which is in line with the preliminary response observed in the study. In addition, thrombocytopenia, the primary safety concern, was predictable, with lower patient platelet nadir values observed with increasing doses and AMG 595-conjugated antibody exposure. Overall, AMG 595 exhibited a favorable pharmacokinetic/pharmacodynamic profile in its target patient population of patients with recurrent GBM.

In this study, evidence of durable clinical response was demonstrated with AMG 595; for the two patients with a partial response, responses lasted slightly over 1 and 1.5 years as of data cutoff, respectively, and one occurred within 4 months of initiating treatment. In particular, one of the patients who obtained a partial response had a near complete response, with more than 95% reduction in tumor size. Moreover, this patient had durations of progression-free survival and overall survival of 1.7 and 3.4 years, respectively, providing further evidence of meaningful clinical benefit. It is possible that EGFRvIII expression was necessary but not sufficient to invoke a response to AMG 595. There are numerous reasons why this may be the case; for example, tumors from different patients may have different levels of blood–brain vascular permeability for AMG 595 or may have different rates of internalization for AMG 595. Additionally, the role that the immune system plays in the mechanism of action for antibody–drug conjugates is not yet understood. It is possible that tumor cell lysis may lead to the stimulation of tumor-infiltrating lymphocytes; however, this was not investigated in this trial.

Despite these unknowns, preliminary responses in this study were comparable with results seen with other anti-EGFRvIII inhibitors [12, 16]. Treatment with rindopepimut in combination with temozolomide resulted in an objective tumor response in 31 of 208 evaluable patients (15%); however, this response rate did not differ from that reported for the control group [16]. Treatment with depatuxizumab mafodotin (formerly ABT-414) resulted in one complete response and two partial responses among 24 patients with recurrent GBM [12]. The activity seen in the present study on AMG 595 further validates EGFRvIII as a therapeutic target for GBM. At present, investigations are ongoing to evaluate targeting EGFRvIII in EGFRvIII-positive glioblastoma using a different construct than an antibody–drug conjugate [17].

This study had several limitations. As mentioned previously, EGFRvIII expression was determined for most patients based on a biopsy sample that was collected at either the time of initial tumor debulking surgery or during a recurrence visit, rather than when the patient was presented for enrollment for this study. Therefore, it cannot be determined what the level of EGFRvIII expression was in patients' tumors at the time of AMG 595 treatment. For future studies, it may be beneficial to augment the IHC test with other assays, such as liquid biopsy for circulating tumor DNA or exosomal RNA analyses, or positron emission tomography-labelled EGFRvIII-specific antibody. Additionally, because of prior surgery in earlier lines of GBM treatment, it was expected that a patient's blood–brain barrier was compromised, thus allowing adequate penetration of the antibody–drug conjugate into the brain, as supported by the encouraging responses observed per Macdonald criteria. However, CNS concentrations of antibody were not measured directly for this analysis, as this was a first-in-human study and such measurements are extremely uncomfortable for patients. The preclinical models predicted that AMG 595 doses with exposures higher than the threshold of approximately 5 µg/mL would likely be efficacious, and according to the observed pharmacokinetics data, doses ≥ 1 mg/kg Q3W surpassed the threshold of 5 µg/mL. Moreover, the responses observed in the study based on stringent Macdonald criteria confirmed all preliminary responses observed at doses ≥ 1 mg/kg.

Results from this study indicated that in patients with recurrent GBM, AMG 595 treatment had a generally acceptable safety profile; however, thrombocytopenia occurred in half of the patients. Although an insufficient percentage of patients experienced a response with AMG 595 to meet the primary objective of this study, there were indications that other endpoints (e.g., survival data) may be informative for ongoing investigations of this tumor target with alternative treatment modalities (e.g., AMG 596). The observation of partial responses suggests that for some patients, a macromolecule, such as an antibody–drug conjugate, may be able to traverse the blood–brain barrier to interact with GBM tumors. Overall, results from this study indicate that AMG 595 is a promising therapy with benefit for some patients with EGFRvIII-mutated GBM who otherwise have limited treatment options.

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Compliance with ethical standards

Conflict of interest Mark Rosenthal has nothing to disclose. Richard Curry has part-time employment as medical director for Bexion Pharmaceuticals with potential stock options. David A. Reardon has participated in advisory boards for AbbVie, Advantagene, Agenus, Bristol-Myers Squibb, Celldex, EMD Serono, Genentech/Roche, Inovio, Merck, Merck KGaA, Monteris, Novocure, Oncorus, Oxigene, Regeneron, Stemline, and Taiho Oncology, Inc; the Dana Farber Cancer Institute received financial compensation for research from Acerta Pharmaceuticals, Agenus, Celldex, EMD Serono, Incyte, Inovio, Midatech, Omniox, and Tragara. Erik Rasmussen is an employee of and stockholder in Amgen Inc. Vijay V. Upreti is an employee of and stockholder in Amgen Inc. and is the Clinical Pharmacology Oncology Therapeutic Area Head at Amgen Inc. Michael A. Damore is a stockholder in Amgen Inc. and was an employee of Amgen Inc. at the time that the study was conducted. Haby A. Henary is an employee of Amgen Inc. John S. Hill is a stockholder in Amgen Inc., an author on a US patent awarded to Amgen related to this work, and was an employee of Amgen Inc. at the time that the study was conceived and initiated. Timothy Cloughesy has consulted for Roche/Genentech, VBL, Merck, Bristol-Myers Squibb, Agios, Boston Biomedical, Tocagen, Deciphera, VBI, Celegene, Puma, and Lilly, and he is a stock option holder for Notable Labs and a board member for Global Coalition for Adaptive Research, a 501(c)3 nonprofit organization.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent All patients provided written informed consent.

Data sharing statement There is a plan to share data. This may include de-identified individual patient data for variables necessary to address the specific research question in an approved data-sharing request; also related data dictionaries, study protocol, statistical analysis plan, informed consent form, and/or clinical study report. Data sharing requests relating to data in this manuscript will be considered after the publication date and (1) this product and indication (or other new use) have been granted marketing authorization in both the US and Europe, or (2) clinical development discontinues and the data will not be submitted to regulatory authorities. There is no end date for eligibility to submit a data sharing request for these data. Qualified researchers may submit a request containing the research objectives, the Amgen product(s) and Amgen study/studies in scope, endpoints/outcomes of interest, statistical analysis plan, data requirements, publication plan, and qualifications of the researcher(s). In general, Amgen does not grant external requests for individual patient data for the purpose of re-evaluating safety and efficacy issues already addressed in the product labelling. A committee of internal advisors reviews requests. If not approved, requests may be further arbitrated by a Data Sharing Independent Review Panel. Requests that pose a potential conflict of interest or an actual or potential competitive risk may be declined at Amgen's sole discretion and without further arbitration. Upon approval, information necessary to address the research question will be provided under the terms of a data sharing agreement. This may include anonymized individual patient data and/or available supporting documents, containing fragments of analysis code where provided in analysis specifications. Further details are available at the following: https://www.amgen.com/science/clinical-trials/clinical-data-transparency-practices/.

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