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Abstract

Background. Serclutamab talirine (Ser-T, formerly ABBV-321) is an antibody-drug conjugate consisting of an antibody (AM-1-ABT-806) directed against activated epidermal growth factor receptor (EGFR) and a pyrrolobenzodiazepine dimer. We investigated Ser-T monotherapy in a phase I, first-in-human, dose-escalation, and dose-expansion study in patients with advanced solid tumors associated with EGFR overexpression.

Methods. Eligible patients (≥18 years) had advanced, histologically confirmed solid tumors associated with EGFR overexpression (centralized testing). Patients received Ser-T intravenously once every 4 weeks (Q4W; 5–50 µg/kg) in the dose-escalation phase. Herein, preliminary antitumor activity at the recommended phase II dose (RP2D) is reported only for patients with glioblastoma ($n = 24$); additional assessments included all treated patients.

Results. Sixty-two patients (median age: 58 years) were enrolled within the dose-escalation ($n = 43$) and dose-expansion ($n = 19$) phases. One dose-limiting toxicity, grade 3 aspartate aminotransferase and alanine aminotransferase elevation, occurred at 20 µg/kg during dose escalation. The Ser-T RP2D regimen of 50 µg/kg × 1 (loading dose) followed by 25 µg/kg Q4W (maintenance dose) was administered during dose expansion. Fatigue (37%) was the only treatment-emergent adverse event (AE) occurring in >25% of patients. Two patients (3%) reported mild treatment-related ocular AEs (eye pruritus). Responses in patients with glioblastoma included 1 partial response (~33 months), 6 stable disease, and 14 progressive disease (not evaluable: $n = 3$).

Conclusions. Ser-T monotherapy at doses up to 50 μg/kg initial dose, followed by 25 μg/kg Q4W demonstrated a tolerable safety profile with minimal antitumor activity observed in patients with glioblastoma. The glioblastoma dose-expansion cohort was closed due to a lack of efficacy (NCT03234712).

Key Points

- Phase I first-in-human trial of Ser-T in advanced solid tumors overexpressing EGFR
- Tolerable safety profile at doses ≤50 μg/kg initial dose, followed by 25 μg/kg Q4W
- Minimal antitumor activity was observed in patients with glioblastoma

Importance of the Study

This is the first study of serclutamab talirine (Ser-T), an antibody-drug conjugate (ADC) targeting epidermal growth factor receptor (EGFR). The study evaluated Ser-T monotherapy in patients with EGFR-overexpressing advanced solid tumors including but not limited to glioblastoma, colorectal cancer, head and neck squamous cell carcinoma, and non-small cell lung cancer. Results of the study demonstrated that Ser-T monotherapy had a tolerable safety profile at doses up to 50 μg/kg initial dose followed by 25 μg/kg every 4 weeks; however, minimal antitumor activity was reported in a cohort of patients with glioblastoma overexpressing EGFR. Although the glioblastoma dose-expansion cohort was closed due to a lack of clinical activity, it is noteworthy that a patient with a prolonged partial response was identified in the study. Research aimed at identifying novel ADCs as well as predictive biomarkers to target patient subpopulations is in progress.

The epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase that mediates intracellular signaling pathways (cell proliferation, survival, differentiation, and migration) and is involved in the pathogenesis and progression of various cancers.[1] Overexpression of EGFR is a notable molecular characteristic of numerous epithelial solid tumors, including glioblastoma (GBM), non-small cell lung cancer (NSCLC), head and neck squamous cell carcinoma (HNSCC), and colorectal cancer (CRC).[2] Alterations in the *EGFR* gene have a high prevalence in GBM and play a significant role in its development, with amplification detected in approximately 50% of tumors.[3] EGFR-targeted therapies have led to improvements in clinical outcomes of patients with cancer[4]; however, despite ongoing progress, limited or no therapeutic efficacy has been displayed thus far in GBM clinical trials.[5] The use of antibody-drug conjugates (ADCs) is an alternative approach to targeting EGFR in GBM, as these agents do not depend on abrogation of signaling to achieve therapeutic effect.

GBM, the most common primary malignant brain tumor in adults, remains an incurable disease with high mortality.[6] Recurrent disease is inevitable after first-line treatment. Patients treated with the current standard of care have median progression-free survival of 7 to 10 months.[6] Therapeutic options for GBM have limited success due to inefficient drug delivery across the blood-brain barrier (BBB), an immunosuppressive tumor microenvironment,

and the development of drug resistance.[7] Thus, molecular characteristics and signaling pathways involved in GBM are areas of active research for the development of targeted therapies.

While depatuxizumab mafodotin (depatux-m, formerly ABT-414), an EGFR-targeting ADC using the ABT-806 antibody, did not prolong overall survival (OS) in patients with newly diagnosed GBM,[8] benefit was demonstrated in some patients with *EGFR*-amplified recurrent GBM.[9,10] The use of ADCs for the treatment of GBM is thus an active area of research.

Serclutamab talirine (Ser-T, formerly ABBV-321) is a next-generation ADC composed of a related EGFR-targeting antibody (ABT-806 affinity matured AM1 antibody; AM-1-ABT-806) conjugated to a pyrrolobenzodiazepine (PBD)-dimer via a maleimidocaproyl-valine-alanine linker.[11,12] Ser-T targets a cryptic EGFR epitope that is exposed when the receptor is in the extended conformation, which may confer tumor-selective binding to cancer cells overexpressing wild-type *EGFR*. Despite the highly potent PBD toxin payload, the tumor-selective binding nature of the compound may confer a wider therapeutic index for on-target toxicities. Once bound, Ser-T is internalized, the linker undergoes proteolytic cleavage, and the cytotoxic PBD is released, causing DNA crosslinks and cell death. Preclinical studies have shown high activity of Ser-T against GBM cell lines and in patient-derived tumor models.[11]

It is noteworthy that Ser-T contains a highly potent PBD-dimer toxin payload whose DNA crosslinking activity is independent of *EGFR*-signaling pathways.[11] Thus, Ser-T has the potential to overcome EGFR signaling pathway resistance. The results of preclinical studies, as well as the structural composition of Ser-T, provided the rationale for clinical investigation across a wide range of tumors likely to overexpress EGFR or its ligands. In addition, PBD is not associated with ocular side effects, such as corneal epitheliopathy, caused by the payload mafodotin, used in earlier-generation ADCs. We report the findings of a phase I first-in-human trial that evaluated the safety, pharmacokinetics (PK), and antitumor activity of Ser-T in patients with advanced solid tumors associated with overexpression of EGFR, including a cohort of GBM patients treated at the recommended phase II dose (RP2D).

Methods

Study Design

This study was a first-in-human, phase I, multicenter, open-label clinical trial of Ser-T monotherapy that consisted of a dose-escalation followed by a dose-expansion phase. The primary objectives of the dose-escalation phase were to determine the maximum tolerated dose (MTD) and RP2D of Ser-T and to assess its PK, toxicity, and safety profile in patients with advanced solid tumors likely to overexpress EGFR or its ligands. The primary objective of the dose-expansion phase was to further evaluate the safety and PK profile of Ser-T at the RP2D in 2 cohorts of patients with EGFR-overexpressing advanced solid tumors (NSCLC and HNSCC) or GBM. Secondary objectives included assessment of Ser-T preliminary antitumor activity at the RP2D in patients with EGFR-overexpressing solid tumors through overall response rate, duration of response (DOR), disease control rate, progression-free survival (PFS), time to progression, and OS estimates. Herein, antitumor activity assessment is reported only for patients with GBM; safety and PK assessments include all treated patients.

The study was conducted in accordance with applicable principles governing ethical and clinical trial conduct, as provided in the Declaration of Helsinki and its later amendments. The trial was registered with ClinicalTrials.gov (NCT03234712) before study initiation and was approved by the independent ethics committee/institutional review board of all participating institutions. Before enrollment, written informed consent was obtained from all patients or their legally authorized representatives.

Patients

This study enrolled adult patients (≥18 years) with histologically or cytologically confirmed solid tumors associated with overexpression of EGFR (including but not limited to CRC, GBM, HNSCC, NSCLC, sarcoma, bladder, cervical, esophageal, or kidney cancers) that had progressed on prior treatment and were not amenable to resection or other approved therapies with curative intent. Patients had measurable disease per Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST v1.1) or Response Assessment in Neuro-Oncology (RANO; for GBM) with a minimum life expectancy of 12 weeks, an Eastern Cooperative Oncology Group performance status of 0–1, and adequate bone marrow, hematologic, hepatic, and renal function. Patients enrolled in the dose-escalation phase were unselected for EGFR overexpression. For the dose-expansion phase, EGFR overexpression in tumor tissue demonstrated by central assessment was required, using an mRNA assay for patients with GBM, and immunohistochemistry (IHC) for patients with non-GBM. The cutoff for selection of patients with GBM was based on EGFR mRNA >50th percentile expression to enrich for patients with higher EGFR expression. Patients with GBM (dose-expansion cohort) were required to have archived diagnostic formalin-fixed paraffin embedded (FFPE) blocks/slides available for biomarker analysis.

Main exclusion criteria included significant cardiac risk factors, active uncontrolled infection (National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAE] v4.03) grade ≥3, known uncontrolled metastases to the central nervous system, major surgery ≤21 days prior to the first dose of Ser-T, any prior exposure to a PBD-containing agent, or significant hepatic steatosis. Patients who had anticancer therapy including chemotherapy, immunotherapy, radiotherapy, hormonal therapy, biologic therapy, or investigational anticancer therapy ≤21 days or herbal anticancer therapy ≤7 days prior to the first dose of Ser-T were excluded. No washout period was required for palliative radiation to bone, skin, or subcutaneous metastases for ≤10 fractions and for patients on erlotinib therapy; a washout period of 5 half-lives was adequate for approved targeted small molecules. Patients with >3 lines of systemic cytotoxic therapy (excluding adjuvant or neoadjuvant therapy) were not eligible for enrollment.

Dose-Escalation and Dose-Expansion Phases

Patients received Ser-T intravenously (IV) once every 4 weeks (Q4W; 5–50 μg/kg) in the dose-escalation phase. A Bayesian continual reassessment methodology was utilized for dose escalation with the objective of defining a relationship between dose and rate of dose-limiting toxicities (DLTs). The continual reassessment methodology utilized a 2-parameter Bayesian logistic regression model incorporating the escalation with overdose control principle for patient safety to estimate MTD for subsequent cohorts using all available data. Dose-escalation steps did not exceed a 100% increase in dose for dose levels <30 μg/kg and did not exceed a 67% increase in dose for dose levels ≥30 μg/kg. Dosing began at 5 μg/kg IV Q4W (Supplementary Figure 1). Any grade ≥3 AE not due to disease progression or any underlying disease was considered a DLT if occurring during the DLT assessment period (first cycle of Ser-T dosing; 28 days). The following grade ≥3 AEs were not considered DLTs: grade 3 nausea, vomiting, or diarrhea (adequately managed or continued for <72 h); grade 3 or 4 neutropenia without fever (dose delay for next treatment cycle ≤7 days); grade ≥3 thrombocytopenia (not requiring supportive care; dose delay for next treatment cycle ≤7 days); grade 3 or 4 lymphopenia

(no clinically significant symptoms); grade 3 or 4 leukopenia (no clinically significant symptoms; dose delay for next treatment cycle <7 days); grade 3 anemia not requiring transfusion (dose delay for next treatment cycle <7 days). The investigator and sponsor also evaluated toxicities occurring after the DLT assessment period.

Initial dose-escalation cohorts enrolled at least 1 DLT-evaluable patient, and thereafter the Bayesian logistic regression model was updated to obtain the recommended dose for the subsequent cohort. When the first DLT or grade 2 treatment-related AE was observed, current and subsequent cohort enrollment was increased to a minimum of 3 DLT-evaluable patients. The MTD was defined as the highest dose level at which ≤33.3% of patients experienced a DLT with a minimum of 6 patients enrolled. The RP2D was not higher than the MTD and was selected on the basis of the type of DLTs observed. The dose-expansion phase further assessed the RP2D at the identified dosing interval in the EGFR-overexpressing patient cohorts. Treatment continued until disease progression (according to RECIST v1.1 or RANO), unacceptable toxicity, or other discontinuation criteria were met. All patients who discontinued treatment were monitored for safety and those who discontinued for reasons other than disease progression were monitored for tumor assessments.

Assessments

AEs, laboratory data, electrocardiograms, physical examinations, and vital signs were assessed throughout the study. AEs were assessed from the time of study drug administration until 60 days following discontinuation of the study drug. AEs were also collected for patients who discontinued treatment prior to progression and were followed for radiographic assessments, with collection occurring per radiographic scan schedule.

Blood samples for PK analysis (Ser-T, total antibody [AM1-ABT-806], unconjugated PBD) were collected at designated time points throughout the study. For the dose-escalation and dose-expansion phases, samples were taken immediately before or after the end of infusion (and 2 h after the start of infusion) on day 1 of all treatment cycles.

Serum analytes of Ser-T and total antibody were quantified using a validated ELISA and plasma concentrations of unconjugated PBD were quantified using liquid chromatography-tandem mass spectrometry. PK parameters, such as maximum observed plasma concentration (C_{max}), time to C_{max} (peak time, T_{max}), terminal elimination half-life ($t_{1/2}$), clearance, volume of distribution, and area under the plasma concentration-time curve from time 0 to the time of last measurable concentration and from time 0 to infinity were calculated using noncompartmental methods.

Tumor assessments (radiographic imaging) were performed within 28 days prior to cycle 1 day 1, then every 2 cycles (±7 days), and at the final visit (±7 days; not required if done within the previous 4 weeks). Tumor assessments continued until disease progression following the same schedule, including in those patients who discontinued treatment prior to progression.

Determination of EGFR Overexpression

Methods for EGFR reverse transcription polymerase chain reaction and amplification by fluorescence in situ hybridization[13] were previously published. An IHC assay was performed on FFPE tissue to assess EGFR protein expression using the EGFR E30 clone (Agilent, Santa Clara, CA) and the Dako EnVision™ FLEX detection system on the Dako Link 48 automated staining platform (Agilent). The E30 clone recognizes both wild-type and *EGFRvIII* forms of EGFR. A trained pathologist evaluated EGFR IHC staining by scoring the percentage of positive cells in neoplastic cells at each intensity, which is categorized into 0 intensity (negative), 1+ intensity (weak), 2+ intensity (moderate), and 3+ intensity (strong).

Statistical Analyses

Descriptive statistics were used to summarize patient baseline characteristics. Safety analyses were performed in patients who received at least 1 dose of the study drug and AE severity was graded according to the NCI CTCAE v4.03 and listed by Medical Dictionary for Regulatory Activities v24.0 system organ class and preferred term. Summary statistics were calculated for each PK sampling time and parameter. Efficacy analyses were performed in patients who received at least 1 dose of the study drug; full analysis set included all patients with at least 1 measurable lesion at baseline. Tumor responses (including progressive disease) were assessed using RANO criteria[14] for patients with GBM (and according to RECIST v1.1 for other solid tumors). Response rate was calculated among patients with measurable disease at baseline and 95% confidence intervals (CIs) were constructed for the estimated overall response rate. PFS duration was defined as the time period from the first dose of Ser-T to the earliest date of disease progression or death, whichever occurred first. All events of disease progression were included irrespective of the study drug discontinuation status. Duration of OS was determined from the time of the first dose of Ser-T to death from any cause. All events of death were included irrespective of study-drug discontinuation status. PFS and OS were analyzed using the Kaplan-Meier method and presented as median time with 95% CIs.

Results

Patient Demographics and Baseline Characteristics

As of June 2021, a total of 62 patients (30 [48%] male, 32 [52%] female; median age 58 years; range: 25–84) were enrolled within dose-escalation (*n* = 43) and dose-expansion (*n* = 19) cohorts. The tumor types included: GBM (*n* = 24), CRC (*n* = 19), NSCLC (*n* = 6), HNSCC (*n* = 5), and other solid tumors (*n* = 8; penile, urothelial, pancreatic, esophageal, gallbladder [1 patient each] and appendiceal [*n* = 2]). Demographics and baseline characteristics are shown in **Table 1**.

Table 1 Demographics and Baseline Clinical Characteristics

Characteristic	Total (N = 62)
Age, median (range)	58 [25–84]
Gender, n (%)	
Male	30 (48)
Female	32 (52)
ECOG performance status, n (%)	
0	25 (40)
1	37 (60)
Cancer type, n (%)	
GBM	24 (39)
CRC	19 (31)
NSCLC	6 (10)
HNSCC	5 (8)
Other	8 (13)
Number of lines of prior systemic therapy, n (%)	
1	6 (10)
2	20 (32)
3	17 (27)
4	5 (8)
≥5	14 (23)
Median time from the initial diagnosis to the first dose of study drug, months (range)	24 [7–144]

CRC, colorectal cancer; ECOG, Eastern Cooperative Oncology Group; GBM, glioblastoma; HNSCC, head and neck squamous cell carcinoma; NSCLC, non-small cell lung cancer.

The median time from the initial diagnosis to the first dose of Ser-T was 24 months (range: 7–144). Fourteen patients (23%) received ≥5 prior lines of systemic therapy; the median was 3 (range: 1–10).

Safety

Median duration of treatment was 29 days (range: 1–437) for the dose-escalation phase, and 29 days (range: 1–225) for the dose-expansion phase. Treatment-emergent (TEAEs) and -related AEs (TRAEs) and associated grade ≥3 events for all patients (dose-escalation and dose-expansion phases) are summarized in Table 2. One DLT of grade 3 aspartate aminotransferase (AST) and alanine aminotransferase (ALT) elevation occurred at 20 μg/kg during dose escalation. Additionally, safety events of interest of moderate to severe thrombocytopenia (2/12) and elevated ALT (3/12) or AST (4/12) were observed after the DLT period during the 50-μg/kg Q4W dose-escalation cohort and typically occurred after the second dose of 50 μg/kg. In view of the above safety observations, the 50-μg/kg Q4W dose-escalation cohort was modified to 50 μg/kg × 1 (loading dose) followed by 25 μg/kg Q4W (maintenance dose), which became the Ser-T RP2D regimen administered to patients in the dose-expansion phase (Supplementary Figure S1).

Fatigue (37%) was the only any-grade TEAE occurring in >25% of patients. Most common grade ≥3 TEAEs (≥10% total patients) were increased AST (13%), increased gamma-glutamyltransferase (GGT), increased ALT (11% each), and thrombocytopenia (10%). Fatigue (29%), increased liver enzymes (GGT, 21%; ALT, 18%; AST, 16%), and thrombocytopenia (18%) were the most frequent (>15% total patients) any-grade treatment-related toxicities observed; grade ≥3 (≥10% total patients) events included increased GGT (11%) and increased AST (10%). Treatment-related ocular AEs (eye pruritus) were reported in only 2 patients (3%). All additional ocular events related to treatment were reported in 1 patient (2%) and included blurred vision, corneal erosion, corneal toxicity, dry eye, and keratitis. TRAEs of infection included conjunctivitis (n = 4, 7%; also considered an ocular AE), and oral candidiasis/fungal infection (n = 3, 5%). Treatment-related conjunctivitis was reported in 4 patients with GBM. Of these, 1 patient reported a grade 2 event (duration: 117 days) and later a grade 1 event that was ongoing at study end. The other 3 patients reported grade 1 events that were ongoing at study end.

TRAEs leading to study drug dose reductions, interruptions, or discontinuations occurred in 3 (5%), 14 (23%), and 5 (8%) patients, respectively. AE counts were not mutually exclusive. Thrombocytopenia accounted for dose reduction in 2 of the 3 patients who had TRAEs that led to dose reduction; increased AST, ALT, and GGT led to dose reduction in the remaining patient. Multiple TRAEs leading to dose interruption were reported in individual patients. These events included increased alkaline phosphatase, ALT, AST, and GGT, fatigue, corneal erosion, thrombocytopenia, peripheral edema, cough, pneumonitis, and nausea. Increased GGT, hepatotoxicity, epistaxis, organizing pneumonia, and thrombocytopenia were TRAEs leading to discontinuation of Ser-T in 5 patients.

Four serious AEs occurred during the study and were considered possibly related to treatment by the investigators: cerebrovascular accident, epistaxis, hepatotoxicity, and thrombocytopenia. Each serious AE was reported in 1 (2%) patient. Death due to TEAEs occurred in 15 patients (24%), resulting from malignant neoplasm progression (n = 11; 18%), epistaxis, pneumonitis (n = 1, 2%; each), and *Pneumocystis jirovecii* infection/pneumonia (n = 2, 3%). Of these, only epistaxis was considered possibly related to Ser-T by the investigator and occurred in a patient with HNSCC who had a necrotic infiltrative tumor involving the pharynx and larynx in close proximity to both internal carotid arteries.

Pharmacokinetics

Complete concentration-time profiles were available from 42 and 14 patients during cycle 1 and cycle 3, respectively. The mean $t_{1/2}$ ranged between 2.4 and 3.1 days for Ser-T and total antibody (Table 3, Supplementary Table S1) and there was a high correlation between the ADC and total antibody exposures (Supplementary Figure S2). Plasma concentrations of PBD were below the lower limit of quantification for all patients at all time points analyzed.

Table 2. Treatment-Emergent and Treatment-Related Adverse Events

| | Ser-T (N = 62) | | | |
| | TEAE | | TRAE | |
	Any grade (≥15% of total)	Grade ≥3 (≥5% of total)	Any grade (≥10% of total)	Grade ≥3 (≥1 patient)
Any, n (%)	62 (100)	47 (76)	51 (82)	16 (26)
Fatigue	23 (37)	1 (2)	18 (29)	0
Nausea	15 (24)	0	9 (15)	0
Increased GGT	15 (24)	7 (11)	13 (21)	7 (11)
Epistaxis	14 (23)	1 (2)	8 (13)	1 (2)
Hypokalemia	14 (23)	3 (5)	0	0
Increased ALT	13 (21)	7 (11)	11 (18)	5 (8)
Increased AST	13 (21)	8 (13)	10 (16)	6 (10)
Thrombocytopenia	13 (21)	6 (10)	11 (18)	4 (7)
Constipation	12 (19)	0	1 (2)	0
Vomiting	11 (18)	2 (3)	3 (5)	0
Cough	10 (16)	0	0	0
Decreased appetite	10 (16)	6 (10)	5 (8)	0
Dyspnea	10 (16)	1 (2)	2 (3)	0
Peripheral edema	10 (16)	0	3 (5)	0
Pyrexia	10 (16)	0	3 (5)	0
Anemia	8 (13)	2 (3)	3 (5)	2 (3)
Increased blood alkaline phosphatase	8 (13)	2 (3)	7 (11)	2 (3)
Increased blood bilirubin	3 (5)	2 (3)	1 (2)	1 (2)
Cerebrovascular accident	1 (2)	1 (2)	1 (2)	1 (2)
Diarrhea	5 (8)	1 (2)	1 (2)	1 (2)
Hepatotoxicity	1 (2)	1 (2)	1 (2)	1 (2)
Decreased lymphocyte count	4 (7)	2 (3)	3 (5)	1 (2)
Stomatitis	1 (2)	1 (2)	1 (2)	1 (2)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase, Ser-T, serclutamab talirine; TEAE, treatment-emergent adverse event; TRAE, treatment-related adverse event.

Exploratory Biomarker and Antitumor Activity in GBM Patients

Biomarker and efficacy assessments were performed on the 24 patients with GBM treated with an initial dose of 50 μg/kg (1 patient at 50 μg/kg Q4W, and 23 at 50 μg/kg × 1 followed by 25 μg/kg Q4W). Tumor EGFR RNA expression was evaluated in GBM tumor specimens from 13 patients treated in the dose-escalation (unselected for EGFR overexpression) and 11 patients from the dose-expansion (selected for EGFR overexpression) cohorts. Among these 24 patients with GBM, 17 (71%) had *EGFR* RNA-expressing tumors. Nine of these 24 (38%) tumors also harbored *EGFRvIII* mutation.

Investigator-reported best overall responses per RANO criteria are summarized in Figure 1. Responses included 1 partial response (PR), 6 stable disease (SD), 14 progressive disease (PD); 3 patients were not evaluable for response. Median duration of clinical benefit (complete response + PR + SD) was 6.4 months (95% CI: 3.0–not reached). The median PFS was 1.8 months (95% CI: 1.3–5.8) and the median OS was 7.1 months (95% CI: 4.1–12.3). A notable durable PR was achieved in a patient with EGFR expression slightly below the chosen cutoff for dose expansion; however, expression was higher than typically observed in cases that harbor *EGFR* amplification.[13] This patient remained on study for 28 months and had an ongoing deep PR for at least 33 months after the first dose of Ser-T (see details in Figure 2 and Supplementary Results).

No clear correlation between EGFR expression by RNA and response was noted in this GBM cohort (Figure 1). Time on the study, response, and EGFR expression levels are presented in Figure 3 for each individual patient.

Discussion

In this study, Ser-T monotherapy up to 50 μg/kg Q4W demonstrated a tolerable safety profile during the DLT

Table 3 PK Parameters of Ser-T and AM-1 S238C (Total Ab) on Cycle 1 or Cycle 3

Dosing	Dose µg/mL	N	C_{max} ng/mL	T_{max}[a] h	AUC_t h*µg/mL	AUC_{inf} h*µg/mL	$t_{1/2}$[b] day	CL[c] mL/h/kg	V_d[d] mL/kg	DN-C_{max} ng/mL per µg/kg	DN-AUC_t h*ng/mL per µg/kg	DN-AUC_{inf} h*ng/mL per µg/kg
Q4W			Ser-T first infusion (cycle 1) intensive PK									
	5	1	68.8	2	2.85	3.33	1.5	1.50	75.5	13.8	570	666
	10	2	174, 177	2, 4	6.57, 7.33	7.92, 9.90	1.7, 2.2	1.01, 1.26	73.4, 75.1	17.4, 17.7	657, 733	792, 990
	20	3	329 (330, 8.0)	2 (2, 4)	15.9 (16.3, 28)	16.9 (17.2, 26)	2.2 ± 0.64	1.19 (1.21, 23)	92.5 (93.0, 12)	16.5 (16.5, 8.0)	795 (814, 28)	843 (860, 26)
	30	5	470 (483, 26)	2 (2, 2)	23.0 (26.9, 74)	24.6 (28.4, 70)	2.0 ± 0.64	1.22 (1.35, 41)	88.6 (89.7, 16)	15.7 (16.1, 26)	766 (896, 74)	819 (948, 70)
	40	5	647 (684, 40)	2 (2, 2)	34.7 (36.4, 34)	36.4 (37.9, 32)	2.6 ± 0.91	1.10 (1.15, 32)	105 (108, 27)	16.2 (17.1, 40)	868 (910, 34)	909 (948, 32)
	50	26	882 (924, 33)	2 (2, 4)	45.3 (47.9, 33)	47.1 (49.7, 32)	2.7 ± 1.4	1.06 (1.13, 39)	107 (115, 41)	17.6 (18.5, 33)	905 (959, 33)	943 (995, 32)
	Overall	42	–	2 (2, 4)[e]	–	–	2.4 ± 1.1	1.10 (1.17, 35)	101 (107, 38)	17.0 (17.7, 31)	854 (913, 38)	906 (961, 36)
Q4W			AM-1 S238C (total Ab) first infusion (cycle 1) intensive PK									
	5	1	69.1	2	4.27	4.81	2.1	1.04	75.2	13.8	855	962
	10	2	184, 194	2, 4	16.2, 9.23	176, 10.1	4.0, 2.1	0.570, 0.995	79.5, 71.5	18.4, 19.4	1620, 923	1760, 1010
	20	3	341 (343, 11)	2 (2, 4)	20.3 (20.7, 25)	21.6 (22.0, 23)	3.0 ± 1.1	0.925 (0.940, 21)	97.8 (99.2, 21)	17.1 (17.1, 11)	1010 (1030, 25)	1080 (1100, 23)
	30	5	449 (465, 31)	2 (2, 4)	26.0 (31.8, 84)	27.9 (33.8, 81)	2.4 ± 0.91	1.07 (1.21, 44)	94.7 (97.7, 26)	15.0 (15.5, 31)	868 (1060, 84)	932 (1130, 81)
	40	5	685 (715, 33)	2 (2, 2)	39.2 (40.9, 33)	41.5 (43.2, 32)	3.3 ± 1.2	0.965 (1.00, 32)	115 (120, 32)	17.1 (17.9, 33)	980 (1020, 33)	1040 (1080, 32)
	50	26	917 (978, 42)	2 (2, 4)	59.9 (70.0, 68)	71.5 (106, 131)	3.3 ± 2.5	0.699 (0.853, 54)	97.9 (107, 43)	18.3 (19.6, 42)	1200 (1400, 68)	1430 (2120, 131)
	Overall	42	–	2 (2, 4)[e]	–	–	3.1 ± 1.8	0.790 (0.922, 47)	97.5 (104, 38)	17.6 (18.5, 38)	1100 (1270, 65)	1270 (1740, 129)

Ab, antibody; AUC, area under the plasma concentration-time curve; AUC_t, AUC from time 0 to time of last measurable concentration; C_{max}, maximum observed plasma concentration; CL, clearance; DN, dose-normalized to cohort dose; PK, pharmacokinetic; Q4W, once every 4 weeks; Ser-T, serclutamab talirine; T_{max}, time to C_{max}; $t_{1/2}$, terminal phase elimination half-life; SD, standard deviation; V_d, volume of distribution.

$N >3$ presented as geometric mean (mean, %CV); $N = 2$ presented individual values; $N = 1$ presented as individual value.

[a]Median (min, max).

[b]Harmonic mean ± pseudo SD.

[c]CL for first infusion is calculated as dose/AUC_{inf}.

[d]Vd is calculated on the basis of respective clearance after the first or third infusion.

[e]$N = 38$, excluded patients ($n = 4$) due to $T_{max} >24$ h (first sampling).

observation window. However, continued dosing at 50 μg/kg revealed toxicities consistent with PBD-dimer toxins, which led to a reduction of the dosing regimen to a loading dose of 50 μg/kg followed by maintenance 25 μg/kg Q4W after cycle 1 day 1. Fatigue, increased liver enzymes, and thrombocytopenia were the most common treatment-related toxicities observed. These were reversible and manageable with dose reductions and interruptions. Epistaxis was the only TEAE that led to death considered possibly related to the effect of Ser-T in a patient with HNSCC that was encroaching into the arteries.

Minimal antitumor activity was observed in the cohort of 24 patients with GBM. One patient achieved a durable PR and 6 patients had SD, providing a clinical benefit rate of 33.3%, but with no clear correlation observed between Ser-T antitumor activity and level of EGFR expression by RNA. The median PFS of 1.8 months and median OS of 7.1 months were comparable with those observed in phase III clinical trials for recurrent GBM.[15] The GBM dose-expansion cohort was subsequently closed due to lack of efficacy.

An insufficient number of patients with non-GBM solid tumor types (5 NSCLC, 2 HNSCC, and 4 others) were dosed with Ser-T at the RP2D to provide meaningful statistical analyses of efficacy. Following the closing of the GBM cohort, the phase I study was terminated by the sponsor due to a change in development strategy.

Although minimal antitumor activity was observed with GBM, it is noteworthy that 1 patient with a prolonged PR was identified in the current study (case details are discussed in the Supplementary Results). This patient remains without disease progression as of September 2022. This observation was similar to other rare "super-responder" cases reported during the development of depatux-m.[8,9] In addition, the results of the INTELLANCE-2 phase II study also support that some patients with GBM will likely receive benefit from treatment with depatux-m in combination with temozolomide in *EGFR*-amplified, recurrent GBM.[10] At this stage in development it is clear that these patients are not identifiable prospectively.

Ser-T was developed to improve on depatux-m, as it demonstrated an increased affinity to EGFR in preclinical studies and contained the more potent PBD-dimer payload. However, this study did not demonstrate a correlation between Ser-T efficacy and *EGFR* expression in patients with GBM. A limitation of this study is that the assessment of EGFR expression status during enrollment screening was done on archived diagnostic FFPE tissue samples rather than fresh tissue samples from recurrent GBM tumors. While there is strong evidence supporting that EGFR status does not change in the majority of recurrent GBM tumors compared to that at primary diagnosis,[16–18] this was not explicitly tested in this study. Thus, the possibility of a change in EGFR expression status contributing to the observed minimal clinical benefit from Ser-T treatment cannot be ruled out.

It is also possible that the dose limitation inherent with using PBD payloads[19] does not allow adequate distribution
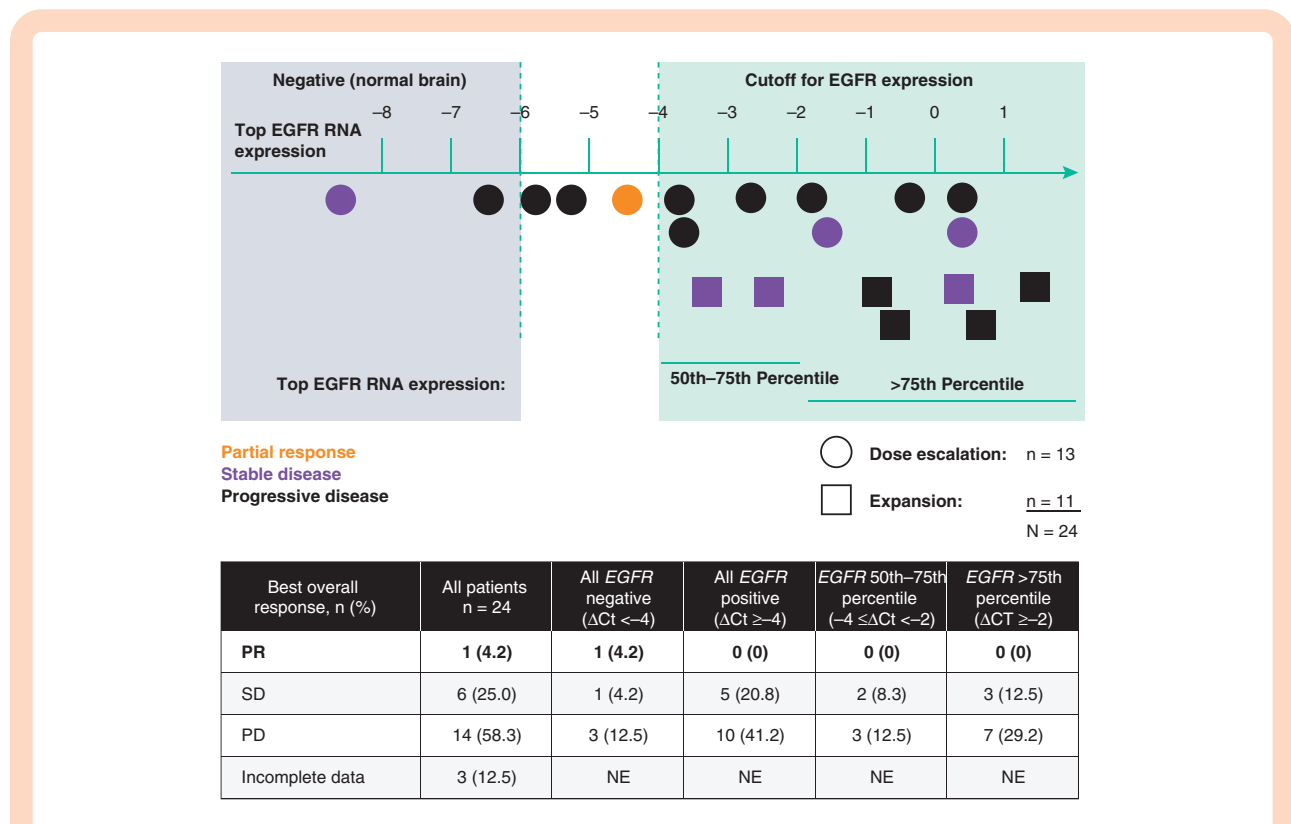
Figure 1. Antitumor response and EGFR RNA expression levels in patients with GBM. ΔCt, change in gene expression; EGFR, epidermal growth factor receptor; GBM, glioblastoma; NA, not available; PD, progressive disease; PR, partial response; SD, stable disease.

Best overall response, n (%)	All patients n = 24	All *EGFR* negative (ΔCt <−4)	All *EGFR* positive (ΔCt ≥−4)	*EGFR* 50th–75th percentile (−4 ≤ΔCt <−2)	*EGFR* >75th percentile (ΔCT ≥−2)
PR	**1 (4.2)**	**1 (4.2)**	**0 (0)**	**0 (0)**	**0 (0)**
SD	6 (25.0)	1 (4.2)	5 (20.8)	2 (8.3)	3 (12.5)
PD	14 (58.3)	3 (12.5)	10 (41.2)	3 (12.5)	7 (29.2)
Incomplete data	3 (12.5)	NE	NE	NE	NE
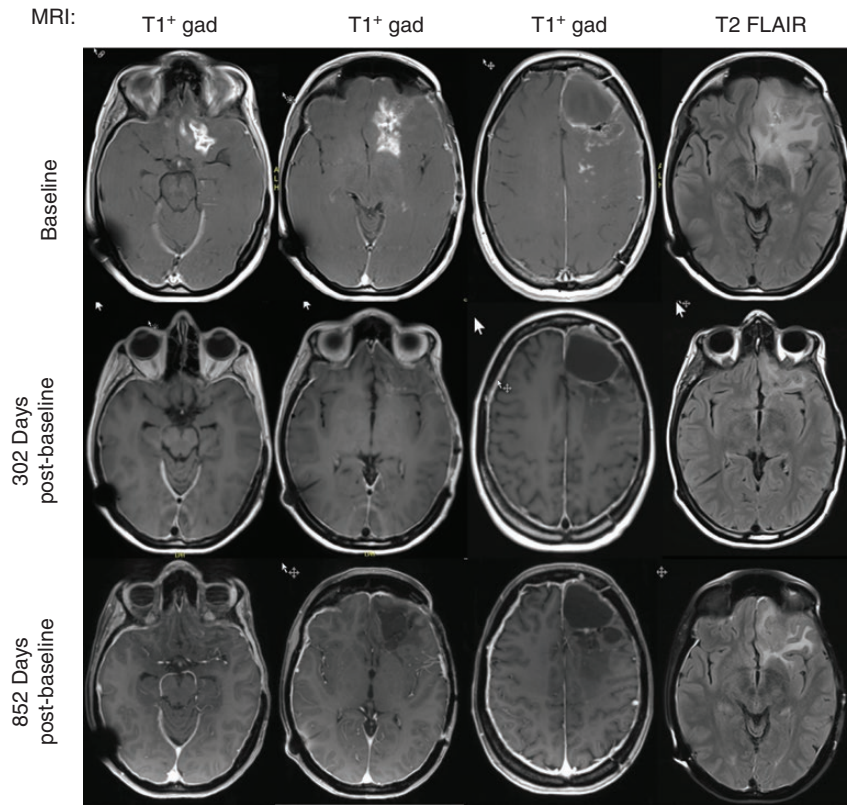
Figure 2. Time on study, response, DOR, and EGFR expression levels in each patient with GBM. ΔCt, change in gene expression; DOR, duration of response; EGFR, epidermal growth factor receptor; GBM, glioblastoma; PR, partial response; SD, stable disease.
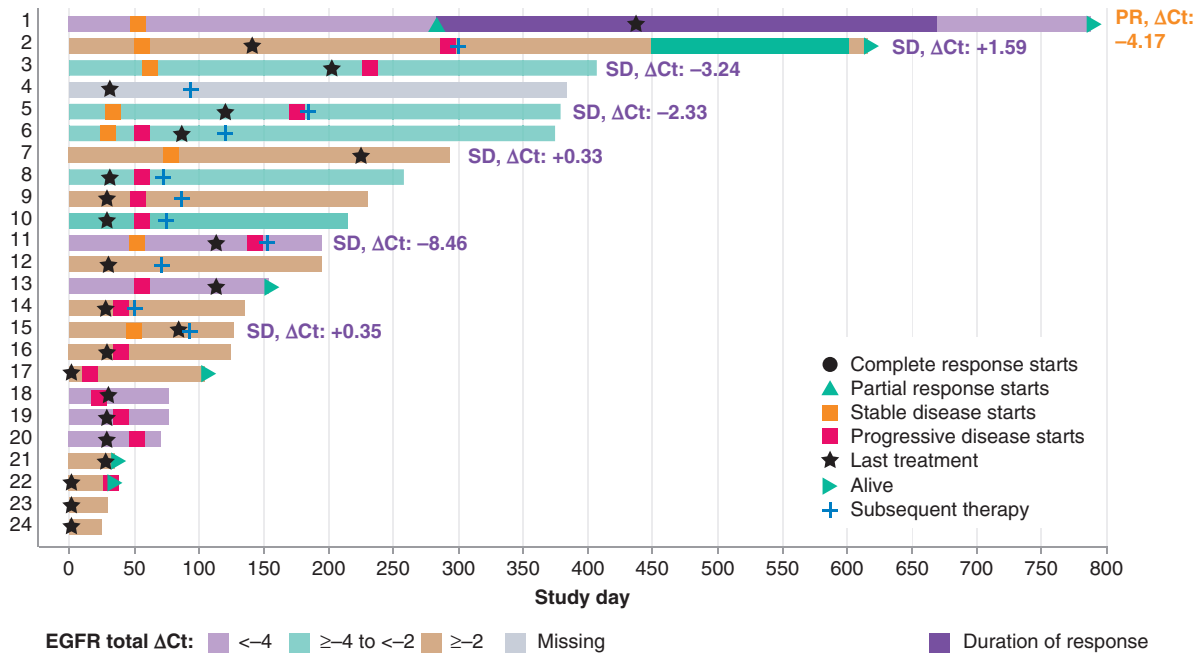
Figure 3. MRI analysis of a patient with GBM and partial response by RANO criteria. gad, gadolinium; FLAIR, fluid-attenuated inversion recovery; RANO, Response Assessment in Neuro-Oncology criteria.

of Ser-T to intracranial tumors. This hypothesis could explain why Ser-T, despite higher EGFR affinity and more-potent payload, did not reproduce or improve the efficacy observed with depatux-m. A recent preclinical study has shown that ADCs that are homogeneous and have a low drug-antibody ratio (DAR; ≤4) are more efficient at crossing the BBB and delivering their toxic payloads to GBM tumors, compared to ADCs that are heterogeneous and have higher DARs.[20] Ser-T was generated by site-specific conjugation of a PBD dimer to the S239C residue on the AM-1-ABT-806 antibody.[11] This method has previously been shown to generate ADCs that are highly homogeneous and have a low DAR of 2.[12] Therefore, we would not attribute the observed lower therapeutic benefit from Ser-T in this study to a lack of homogeneity or to drug overloading affecting its BBB permeability.

Nonetheless, 1 patient with recurrent GBM did respond to Ser-T treatment, suggesting potential activity in GBM, and highlighting the need to identify the subpopulations that may respond to specific targeted therapies in this disease. Also, as discussed above, the need for dose reductions or interruptions in this study may also have reduced the potential for therapeutic benefit from Ser-T. While EGFR-targeted therapies, such as Ser-T, seem to be facing unique challenges in the treatment of GBM, including the potential reduction of tissue distribution by the BBB, ADCs targeting EGFR may still hold potential for other EGFR-overexpressing systemic solid tumors such as NSCLC and HNSCC.

In conclusion, for GBM, targeted therapy remains a valid approach for investigation and EGFR remains an attractive target for ADCs. Further research is needed to optimize the design and testing of ADCs with the ability to penetrate the BBB while carrying payloads delivering less systemic toxicity than PBD-dimers, and to identify predictors of response for target patient subpopulations.

Supplementary material

Supplementary material is available online at *Neuro-Oncology Advances* online.

Keywords

antibody-drug conjugate | EGFR | glioblastoma | phase I | serclutamab talirine

Data Sharing Statement

AbbVie is committed to responsible data sharing regarding the clinical trials we sponsor. This includes access to anonymized, individual and trial-level data (analysis data sets), as well as other information (e.g., protocols and Clinical Study Reports), as long as the trials are not part of an ongoing or planned regulatory submission. This includes requests for clinical trial data for unlicensed products and indications. These clinical trial data can be requested by any qualified researchers who engage in rigorous, independent scientific research, and will be provided following review and approval of a research proposal and Statistical Analysis Plan (SAP) and execution of a Data Sharing Agreement (DSA). Data requests can be submitted at any time and the data will be accessible for 12 months, with possible extensions considered. For more information on the process, or to submit a request, visit the following link: https://www.abbvie.com/our-science/clinical-trials/clinical-trials-data-and-information-sharing/data-and-information-sharing-with-qualified-researchers.html.

Authorship statement

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