Trotabresib, an oral potent bromodomain and extraterminal inhibitor, in patients with high-grade gliomas: a phase I, "window-of-opportunity" study

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Conflict of Interest

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Abstract

Background: The bromodomain and extraterminal protein (BET) inhibitor trotabresib has demonstrated antitumor activity in patients with advanced solid tumors, including high-grade gliomas. CC-90010-GBM-001 (NCT04047303) is a phase I study investigating the pharmacokinetics, pharmacodynamics, and CNS penetration of trotabresib in patients with recurrent high-grade gliomas scheduled for salvage resection.

Methods: Patients received trotabresib 30 mg/day on days 1–4 before surgery, followed by maintenance trotabresib 45 mg/day 4 days on/24 days off after surgery. Primary endpoints were plasma pharmacokinetics and trotabresib concentrations in resected tissue. Secondary and exploratory endpoints included safety, pharmacodynamics, and antitumor activity.

Results: Twenty patients received preoperative trotabresib and underwent resection with no delays or cancellations of surgery; 16 patients received maintenance trotabresib after recovery from surgery. Trotabresib plasma pharmacokinetics were consistent with previous data. Mean trotabresib brain tumor tissue:plasma ratio was 0.84 (estimated unbound partition coefficient [K_{PUU}] 0.37), and modulation of pharmacodynamic markers was observed in blood and brain tumor tissue. Trotabresib was well tolerated; the most frequent grade 3/4 treatment-related adverse event during maintenance treatment was thrombocytopenia (5/16 patients). Six-month progression-free survival was 12%. Two patients remain on treatment with stable disease at cycles 25 and 30.

Conclusions: Trotabresib penetrates the blood–brain-tumor barrier in patients with recurrent high-grade glioma and demonstrates target engagement in resected tumor tissue. Plasma pharmacokinetics, blood pharmacodynamics, and safety were comparable with previous results for trotabresib in patients with advanced solid tumors. Investigation of adjuvant trotabresib + temozolomide and concomitant trotabresib + temozolomide + radiotherapy in patients with newly diagnosed glioblastoma is ongoing (NCT04324840).

Key words: Trotabresib; glioblastoma; pharmacokinetics; pharmacodynamics; blood–brain-tumor barrier

Key points

- Trotabresib showed notable brain tumor tissue penetration relative to plasma concentrations
- Trotabresib demonstrated target engagement in resected brain tumor tissue
- Safety data support further investigation of trotabresib in high-grade glioma

Importance of the study

Many therapies investigated in gliomas and other central nervous system malignancies have proved ineffective for various reasons, including limited brain tumor tissue penetration. The bromodomain and extraterminal (BET) inhibitor trotabresib has demonstrated single-agent activity in patients with high-grade gliomas, suggesting brain tumor tissue penetration. Here, we investigated trotabresib concentrations in brain tumor tissue following presurgical administration in patients with recurrent high-grade gliomas scheduled to undergo salvage resection. Trotabresib was detectable in resected tissue, and relative concentrations in resected brain tumor tissue and time-matched plasma samples, as well as the estimated unbound partition coefficient, suggested notable brain tumor tissue penetration. Pharmacodynamic analyses demonstrated target engagement in blood and brain tumor tissue. Safety was consistent with previous results in patients with advanced solid tumors. Based on the accumulated body of favorable safety, efficacy, pharmacokinetic, and pharmacodynamic data, a phase Ib/II clinical trial is currently investigating trotabresib in combination with adjuvant temozolomide and radiotherapy in patients with newly diagnosed glioblastoma (NCT04324840).

Introduction

Gliomas are aggressive primary malignant brain tumors that account for the majority of malignant primary central nervous system (CNS) neoplasms in the US and Europe.[1,2] The overall annual incidence of gliomas is approximately 6 per 100,000 population in the US and 5.4 per 100,000 population in Europe.[1,2] The most frequently occurring glioma is glioblastoma, making up 56% of glioma cases, with high-grade astrocytomas accounting for a further 20% of diagnoses.[1] The standard first-line treatment for high-grade gliomas is maximal surgical resection with adjuvant chemoradiotherapy;[3,4] however, even complete resection of high-grade gliomas is unlikely to be curative due to tumor infiltration of surrounding brain tissue, and tumors eventually recur in all patients.[5] The prognosis for patients with recurrent disease remains very poor, with most reports of median overall survival (OS) ranging from 3 to 12 months.[5-7]

Effective treatment of gliomas is challenging for a number of reasons, with limited CNS penetration of therapeutics being one of the most important contributing factors.[8] Diffusion of drugs through the blood–brain barrier (BBB) is restricted to lipid-soluble compounds with a molecular weight < 400 Daltons,[9] and expression of drug efflux transporters on vascular endothelial cells provides an additional barrier to tissue penetration of molecules that have the necessary properties for diffusion.[10] Gliomas and other brain tumors have, however, been suggested to physically disrupt the BBB, resulting in localized increases in BBB permeability. Such areas of increased permeability can be visualized on MRI through the use of contrast agents that selectively enhance tumor tissue.[11] Importantly, disruption of the BBB shows considerable intratumoral heterogeneity, and most glioblastomas also have non-enhancing areas that represent tumor infiltration of brain tissue where the BBB is sufficiently intact to prevent contrast uptake.[11]

Bromodomain and extraterminal (BET) proteins are epigenetic readers that regulate the expression of a variety of genes involved in cancer cell proliferation, survival, and oncogenic progression.[12-17] In particular, the BET protein BRD4 is overexpressed in gliomas relative to healthy tissue, supporting its

investigation as a treatment target.[18] BET inhibitors have shown antitumor activity in preclinical models of glioblastoma,[18-20] and several BET inhibitors have demonstrated BBB penetration in mice.[18-21] However, the relevance of animal data to clinical efficacy in glioblastoma is unclear; the BET inhibitor OTX015 showed antitumor activity and high penetration of brain tumor tissue in a murine orthotopic glioblastoma model, but was present at markedly lower concentrations in peritumoral and distant brain tissue.[20] A subsequent dose-finding study in patients with glioblastoma was stopped due to lack of efficacy,[22] an outcome which may suggest poor brain tumor tissue penetration of OTX015 in humans.

Trotabresib (CC-90010, BMS-986378) is an oral, potent, and reversible small-molecule BET inhibitor that has been shown to reduce tumor growth in cell line and xenograft models of glioma and other malignancies (data on file). Trotabresib monotherapy 45 mg/day 4 days on/24 days off per 28-day cycle was well tolerated and demonstrated antitumor activity in heavily pretreated patients with advanced cancers, including high-grade gliomas, with prolonged progression-free survival (PFS) up to 3 years in some patients.[23,24] Importantly, trotabresib has physicochemical properties consistent with BBB penetration, including a molecular weight of 383 Daltons and high lipophilicity, and trotabresib concentration–time profiles in plasma and brain were comparable in animal models (data on file). Together, these data provide sufficient supporting evidence to evaluate trotabresib brain tumor tissue penetration in patients with high-grade gliomas, the first such study for a BET inhibitor.

Here, we present the results of CC-90010-GBM-001 (NCT04047303), a phase I "window-of-opportunity" study to evaluate brain tumor tissue penetration, plasma pharmacokinetics (PK), pharmacodynamics, safety, and antitumor activity of trotabresib in patients with recurrent high-grade gliomas who were scheduled to undergo salvage resection.

Materials and methods

Study design

CC-90010-GBM-001 enrolled patients with recurrent high-grade gliomas who were candidates for salvage resection. Patients received trotabresib monotherapy 30 mg/day on days 1–4 prior to salvage resection, which was planned for 6–24 hours after the day 4 dose of study drug. The design of the study is shown in **Supplementary Figure 1**. A dose of 30 mg/day was selected for the preoperative treatment period to maintain platelet count above the minimum of 100,000/mL recommended for surgery, based on previous data showing that this dose level provides a balance of target engagement with a minimal impact on platelet count.[23]

Upon recovery from surgery and ≥ 4 weeks after the first preoperative dose of trotabresib, patients initiated maintenance treatment with trotabresib monotherapy 45 mg/day 4 days on/24 days off in each 28-day cycle, which was the recommended phase II dose and schedule for trotabresib monotherapy identified in the CC-90010-ST-001 study.[23,24] Maintenance treatment continued until disease progression, unacceptable toxicity, or withdrawal of consent.

Patient selection

Eligible patients were ≥ 18 years of age, had progressive World Health Organization grade II diffuse astrocytoma, grade III anaplastic astrocytoma, or grade IV glioblastoma in radiographically confirmed first or second recurrence, and were candidates for salvage resection. Disease progression was defined as either a > 25% increase in the largest bidimensional product of enhancement or a new enhancing lesion for patients with glioblastoma, or a > 25% increase in the largest bidimensional product of enhancement, a new enhancing lesion, or a > 25% increase in a T2 or fluid-attenuated inversion recovery (FLAIR) non-enhancing lesion for patients with grade II or grade III astrocytoma. Patients must have had an Eastern Cooperative Oncology Group performance status (ECOG PS) of 0 or 1, previously completed a course of standard or hypofractionated

radiotherapy at least 12 weeks prior to the first dose of study drug, and have archival formalin-fixed paraffin-embedded (FFPE) tumor tissue suitable for genetic testing.

Key exclusion criteria included anticancer therapy within 4 weeks (6 weeks for nitrosoureas) or 5 half-lives prior to starting trotabresib, major surgery within 4 weeks or minor surgery within 2 weeks prior to starting trotabresib, evidence of CNS hemorrhage on baseline MRI or CT scan, and requirement for increasing doses of corticosteroids to treat symptomatic cerebral edema within 7 days before study entry.

Additional exclusion criteria included mild or asymptomatic SARS-CoV-2 infection within 10 days, or severe/critical SARS-CoV-2 infection within 20 days prior to the first dose of study drug. Acute symptoms must have resolved without sequelae that would place the patient at increased risk while receiving study treatment, based on investigator assessment and consultation with the study medical monitor. Patients who had received a SARS-CoV2 vaccine within 14 days prior to starting study drug were also excluded.

All patients provided written informed consent; the study was conducted in compliance with the International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Good Clinical Practice, and general ethical principles outlined in the Declaration of Helsinki, and was approved by the Institutional Review Board or Ethics Committee of each participating center.

Endpoints and assessments

The primary endpoints of the study were to measure the concentration of trotabresib in resected brain tumor tissue at a single time point following oral dosing (including within contrast-enhancing and non-enhancing tumor, wherever possible) and to evaluate trotabresib plasma PK. Trotabresib concentrations in resected tumor were also compared with trotabresib concentrations in matched plasma samples collected at the same time point during surgery. Safety was assessed as a secondary

endpoint. Exploratory endpoints included antitumor activity, PFS, trotabresib concentration in cerebrospinal fluid (CSF), and pharmacodynamic markers of target engagement.

Tumor tissue for PK and pharmacodynamic analysis and CSF samples for PK analysis were collected during surgery. Plasma samples for assessment of trotabresib PK and pharmacodynamics were obtained at multiple time points throughout the first treatment cycle, and at the time of tumor tissue and CSF sample collection. Trotabresib concentrations in plasma samples were determined by liquid chromatography with tandem mass spectrometry (High Assay Range). PK parameters were calculated by non-compartmental analysis using Phoenix Version 8.3.3.33. Plasma PK parameters assessed included peak plasma concentration (C_{max}), time to peak plasma concentration (t_{max}), area under the plasma concentration–time curve (AUC), and terminal half-life ($t_{½}$). Adverse events (AEs) were classified using Medical Dictionary for Regulatory Activities (MedDRA) version 24.0 and graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 5.0.[25] AEs were reported separately for preoperative treatment with trotabresib 30 mg/day on days 1–4 (from initiation of preoperative treatment to initiation of postoperative maintenance) and postoperative maintenance treatment with trotabresib 45 mg/day 4 days on/24 days off (from the time of initiation of postoperative maintenance therapy onwards). Pharmacodynamic markers investigated included *CCR1* expression in blood and *HEXIM1* expression in blood, FFPE resected on-treatment tumor tissue and FFPE archival tumor tissue. Gene expression was assessed using RNA-Seq. Details of blood and tumor PD biomarker analyses are listed in **Supplementary Materials and Methods**. Tumor assessments by MRI were performed within 24–72 hours post-surgery to serve as a baseline for subsequent evaluations, and at the end of every other treatment cycle from cycle 2 onwards. Antitumor activity in terms of tumor response was assessed in patients with subtotal resection using the appropriate Response Assessment in Neuro-Oncology (RANO) criteria for high-grade or low-grade glioma.[26,27] No formal statistical power calculations were performed.

Results

Patients and treatment

A total of 20 patients were enrolled between January 13, 2020 and February 5, 2021 and received preoperative trotabresib monotherapy 30 mg/day on days 1-4. Four patients did not enter the postoperative treatment period, of whom 2 died of surgical complications (described in detail in the safety section), 1 withdrew from the study, and 1 had progressive disease (PD). The remaining 16 patients initiated postoperative maintenance treatment with trotabresib monotherapy 45 mg/day 4 days on/24 days off. At the time of data cutoff (March 8, 2022), 14 patients had discontinued treatment due to PD and treatment was ongoing in 2 patients, both of whom remained on treatment as of the time of manuscript preparation (November 1, 2022).

Baseline patient demographics and characteristics are shown in **Table 1.** Median age was 47 years, 14 patients (70%) were male and 6 (30%) female, and 12 patients (60%) had an ECOG PS of 0. Nineteen patients (95%) had glioblastoma and 1 patient had progressive diffuse astrocytoma. The enrolled population was heavily pretreated; all patients had previously received radiotherapy and 16 (80%) had received 2 or more previous systemic therapies. One patient had previously received bevacizumab. *MGMT* promoter was methylated in 7 patients (35%) and unmethylated in 7 patients (35%). *MGMT* promoter methylation status was unknown in 6 patients (30%); some study sites did not determine this parameter prior to patient enrollment, as it was not an inclusion criterion for the study, and as all patients had previously progressed during prior treatment with temozolomide, no further treatment with temozolomide was planned. *IDH* mutation status was wild-type in 14 patients (70%), mutant in 5 patients (25%), and not otherwise specified in 1 patient (5%).

Pharmacokinetics (PK)

The plasma PK of trotabresib monotherapy 30 mg/day 4 days on/24 days off in CC-90010-GBM-001 was consistent with the PK of trotabresib monotherapy at this dose and schedule in the CC-90010-ST-001 study (**Figure 1**).[23] On cycle 1, day 1 (C1D1), geometric mean (GM) C_{max} was 392 ng/mL and GM AUC from 0 to 24 hours ($AUC_{0–24}$) was 5083 ng·h/mL. On cycle 1, day 4 (C1D4), GM C_{max} was 720 ng/mL and GM $AUC_{0–24}$ was 11,250 ng·h/mL. Median t_{max} was 1.5 hours on C1D1 and 1.9 hours on C1D4, and mean $t_{1/2}$ was 46 hours on C1D4.

The median time from the day 4 dose of trotabresib to resection was 23 hours (range, 4.6–31.3). Trotabresib was found to penetrate brain tumor tissue; GM trotabresib concentrations in plasma samples collected at the time of surgery and resected brain tumor tissue were 1.02 μM and 0.74 μM, with a mean tumor tissue:plasma ratio of 0.84 (**Figure 2**). GM free trotabresib concentration in brain tissue was calculated to be 0.028 μM. Based on the plasma protein binding and the tissue binding of trotabresib, the estimated unbound partition coefficient (K_{PUU}) value[28] for trotabresib is 0.37. GM trotabresib concentration in CSF was 0.14 μM, and the mean CSF:plasma ratio was 0.17.

Pharmacodynamics

Trotabresib monotherapy showed encouraging target engagement based on modulation of two pharmacodynamic markers of BET inhibition, *HEXIM1* and *CCR1* expression.[29,30] BET inhibition has been shown to decrease *CCR1* expression in blood.[29] *HEXIM1* expression, which is mechanistically linked to BET function, has been shown to increase in response to BET inhibition in blood and tumor tissue.[30] In CC-90010-GBM-001, blood levels of *CCR1* mRNA decreased to 45.8% (standard deviation ± 28.5) of baseline levels after the fourth preoperative dose of trotabresib (**Figure 3A**), consistent with data from patients treated with trotabresib on the same dose and schedule in the CC-90010-ST-

001 study.[23] Blood levels of *HEXIM1* mRNA were increased from baseline in all patients at 72–96 hours after the first preoperative dose of trotabresib (**Figure 3B**)**.** Analysis of *HEXIM1* expression in blood samples obtained at the time of resection showed a relationship between the magnitude of increase in *HEXIM1* expression in blood and trotabresib plasma concentration (**Figure 3C**)**.** A total of 18 patients had sufficient archival tumor tissue available for RNAseq analysis. Comparison of *HEXIM1* expression in FFPE resected brain tumor tissue with expression in archival tissue showed that *HEXIM1* mRNA was increased in 15 of 18 patients (group $P = 0.00093$) (**Figure 3D**).

Safety

The safety profile of trotabresib was consistent with previous reports of trotabresib monotherapy.[23] Any-grade treatment-related AEs (TRAEs) were reported in 12 patients (60%) following preoperative treatment with trotabresib 30 mg/day on days 1–4, of whom 2 (10%) had grade 3/4 TRAEs (ALT increased in 1 patient and lymphopenia in 1 patient; **Figure 4A**). Grade 1/2 thrombocytopenia was reported in 5 (25%) patients during the preoperative period. TRAEs were reported in all 16 (100%) patients who received postoperative maintenance trotabresib 45 mg/day 4 days on/24 days off, with grade 3/4 TRAEs reported in 6 (38%) patients (thrombocytopenia in 5 patients and asthenia in 1 patient; **Figure 4B**). No serious TRAEs were reported.

No patients had surgery delayed or cancelled due to an AE, and no patients required dose modifications for the management of AEs during the preoperative period. During the postoperative adjuvant treatment period, 3 patients had dose interruptions due to AEs (thrombocytopenia in 2 patients and pancreatitis in 1 patient), and 1 patient had a dose reduction due to thrombocytopenia. No patients discontinued treatment due to AEs.

Two patients died of surgical complications unrelated to study drug (intracranial hemorrhage). The first patient, a 40-year-old man, had an uneventful surgery and initial recovery period. On study day 7, 2 days after surgery, a postoperative hematoma was identified that required reoperation. The hematoma was accompanied by a suspected TRAE of grade 1 thrombocytopenia, with a decrease in

platelet count to ~100,000/mL. Reoperation was complicated by excessive bleeding and hydrocephalus requiring external ventricular drainage; the patient subsequently developed a gram-negative infection that was unresponsive to antibiotics on the third day after reoperation and died on study day 15. The second patient had a distant intracranial hemorrhage on study day 5; however, his platelet count and coagulation studies were normal. The patient's recovery was complicated by confirmed SARS-CoV-2 infection on study day 9; the patient had not been vaccinated against SARS-CoV-2 and died on study day 12 following severe respiratory deterioration.

Efficacy

At the March 8, 2022 data cutoff, median PFS was 1.9 months (95% CI 1.4–3.4) and the 6-month PFS rate was 12% (95% CI 2.1–31.5). Subgroup analysis found that patients with *IDH* wild-type disease had a median PFS of 3.0 months (95% CI 1.4–3.6) and a 6-month PFS rate of 16.3% (95% CI 2.7–40.4), while patients with *IDH*-mutant disease had a median PFS of 1.2 months (95% CI 0.4–1.9) and a 6-month PFS rate of 0% (95% CI 0–0). Treatment was ongoing in 2 patients at the time of manuscript submission, both of whom had *IDH* wild-type disease and a methylated *MGMT* promoter. The patients remained on treatment with stable disease as of November 1, 2022 at cycles 25 and 30, representing approximately 23 and 27 months, respectively (**Figure 5**). Of the 17 patients who were evaluable for response, 7 (41%) had stable disease and 10 (59%) had PD per RANO criteria.

Discussion and conclusions

Despite considerable research to identify new therapies for the treatment of glioma, translating antitumor activity in preclinical models of glioma to clinical efficacy has proved challenging, with poor penetration of brain tumor tissue by anticancer agents being a key limitation.[31] The BET protein BRD4 is frequently overexpressed in gliomas, and represents a potential target for anticancer therapy due to the broad-ranging role of BET proteins in tumor growth and survival.[12-18]

The present “window-of-opportunity” study in patients undergoing surgical resection for recurrent high-grade gliomas was a rare opportunity to evaluate drug penetration and changes in pharmacodynamic markers of target engagement in brain tumor tissue in a clinical setting. Our results, which confirm the presence of pharmacologically active drug concentrations in resected glioma tissue, are the first such clinical data for a BET inhibitor. Combined with results from the CC-90010-ST-001 study, which showed that trotabresib monotherapy was well tolerated and had durable antitumor activity in patients with recurrent high-grade gliomas, these findings support further evaluation of trotabresib in this tumor type.[23,24]

The plasma PK profile of trotabresib in the current study was consistent with that seen with the same dose and schedule in CC-90010-ST-001, with comparable C_{max}, t_{max}, and AUC.[23,24] These data confirm that the PK profile of trotabresib in patients with high-grade gliomas is comparable to that seen in patients with advanced solid tumors. Trotabresib was detected in resected brain tumor tissue, and relative concentrations in time-matched brain tumor tissue and plasma samples were suggestive of notable brain tumor tissue penetration, with a mean tissue:plasma ratio of 0.84. The median time from the last dose of trotabresib to surgery was 23 hours (range, 4.6–31.3), and it may be reasonable to suggest that tissue concentrations of trotabresib would be higher at plasma t_{max} (1.9 hours post-dose on day 4) if the brain serves as a rapidly equilibrating compartment. Based on the plasma protein binding and the tissue binding of trotabresib, the estimated unbound partition coefficient (K_{PUU}) value[28] for trotabresib is 0.37. Taken together with the absolute concentration in tumor tissue, the relative concentrations in tissue and plasma, the observed biomarker activity in tumor tissue, and the potency of trotabresib, this K_{PUU} value supports that trotabresib has sufficient penetration into brain tumor tissue to drive pharmacological activity, potentially leading to clinical effects.

Selection of the 45 mg/day 4 days on/24 days off dose and schedule for trotabresib monotherapy in the CC-90010-ST-001 first-in-human study has been described previously.[23,24] Due to the long half-

life of trotabresib (mean $t_{1/2}$ was 46 hours in this present study), the 4 days of consecutive dosing leads to drug accumulation in circulation, which in turn is expected to drive drug penetration into tumor tissue as observed in this study, while the 24-day dosing holiday allows patients to recover. In addition, the 4 days on/24 days off schedule allows trotabresib dosing to be aligned with the standard dosing schedule for temozolomide (days 1–5 of each 28-day cycle).[32-35]

Trotabresib was shown to modulate pharmacodynamic markers of target engagement in blood, based on expression of *CCR1* and *HEXIM1*. Blood levels of *CCR1* mRNA were decreased to 45.8% (standard deviation ± 28.5) of baseline levels after the fourth dose of trotabresib 30 mg/day, consistent with results at the same dose level in the CC-90010-ST-001 study.[23,24] Reductions in blood *CCR1* mRNA to ≤ 50% of baseline levels has previously been associated with plasma BET inhibitor concentrations and response in patients with lymphoma.[36] Trotabresib also increased blood levels of *HEXIM1* mRNA, a marker of target engagement that has a mechanistic link with BET inhibition,[30] with increases observed in all 19 evaluable patients from 72 to 96 hours. Analysis of *HEXIM1* levels in blood samples obtained at the time of surgery showed that the magnitude of increase in *HEXIM1* mRNA expression was associated with trotabresib concentration in time-matched plasma samples. Comparison of *HEXIM1* expression in FFPE on-treatment resected tissue versus archival tumor tissue showed increased *HEXIM1* expression in 15 of 18 samples, indicating that trotabresib reaches pharmacologically active tumor tissue concentrations after 4 days of treatment at the 30 mg/day dose level. Although *CCR1* mRNA levels were measured in brain tumor tissue, *CCR1* expression has not been validated as marker of BET inhibitor target engagement in tumor tissue. *CCR1* mRNA levels in tumor tissue were found to be much lower than those of *HEXIM1* mRNA, and therefore, interpretation of the effects of trotabresib on modulation of *CCR1* expression in brain tumor tissue was not reliable.

The safety profile of trotabresib monotherapy was also comparable to that seen in the CC-90010-ST-001 study,[23,24] with hematological and gastrointestinal TRAEs the most frequently reported. The

majority of TRAEs were mild or moderate in severity, and most individual TRAEs were reported during maintenance treatment with trotabresib 45 mg/day 4 days on/24 days off. Grade 3/4 TRAEs were reported in 2 of 20 (10%) patients following preoperative treatment (ALT increase in 1 patient and lymphopenia in 1 patient), and 6 of 16 (38%) patients during maintenance treatment (thrombocytopenia in 5 patients and asthenia in 1 patient). No patients had surgery delayed or cancelled, and no patients discontinued postoperative maintenance treatment due to AEs. Two patients died of surgical complications unrelated to study drug. The first patient had a postoperative hematoma that required reoperation, and subsequently developed a postoperative gram-negative infection that was unresponsive to antibiotic therapy. The second patient had confirmed SARS-CoV-2 infection that resulted in severe respiratory deterioration; of note, SARS-CoV-2 vaccines had not been approved in Europe at the time of the patient's participation in the study, and thus the patient had not been vaccinated. Although the patient had a postoperative intracranial hemorrhage, their platelet count was normal throughout the study period and the hemorrhage was not considered to be related to treatment with trotabresib.

The 6-month PFS rate in CC-90010-GBM-001 was 12%, with a median PFS of 1.9 months; this result reflects the poor prognosis for the population enrolled in our study, with the evaluable population comprising patients with heavily pretreated recurrent glioblastoma. Although a subgroup analysis based on *IDH* mutation status showed longer PFS in patients with wild-type vs mutant tumors, this result must be interpreted with caution due to the small number of patients—only 5 patients with *IDH*-mutant tumors were enrolled, of whom 2 died of postoperative complications and one withdrew from the study shortly after surgery. Outcomes in subgroups based on *MGMT* promoter methylation status were not evaluated due to the small number of patients with confirmed promoter methylation status (n = 14). Two patients with stable disease remained on treatment with trotabresib maintenance therapy at cycles 25 and 30 as of November 1, 2022, both of whom had *IDH* wild-type tumors and a methylated *MGMT* promoter.

In conclusion, trotabresib demonstrated brain tumor tissue penetration in patients with recurrent high-grade glioma, and a robust PK profile consistent with a previous study in patients with advanced malignancies.[23,24] Trotabresib was also well tolerated and showed modulation of pharmacodynamic markers of target engagement in blood, consistent with previous results.[23,24] Modulation of *HEXIM1* expression in FFPE on-treatment resected tissue suggests that trotabresib concentrations in glioma tissue were sufficient for pharmacological activity. Two patients remain on treatment with prolonged stable disease. Based on these findings and the efficacy of trotabresib in patients with recurrent high-grade gliomas in the CC-90010-ST-001 study,[23,24] a phase Ib/II study, CC-90010-GBM-002 (NCT04324840), is investigating the safety and efficacy of concomitant trotabresib in combination with temozolomide and radiotherapy and adjuvant trotabresib plus temozolomide in patients with newly diagnosed glioblastoma.[37,38]

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References

1. Ostrom QT, Gittleman H, Liao P, et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2010-2014. *Neuro Oncol.* 2017;19(suppl_5):v1–v88.
2. Crocetti E, Trama A, Stiller C, et al. Epidemiology of glial and non-glial brain tumours in Europe. *Eur J Cancer.* 2012;48(10):1532–1542.
3. Weller M, van den Bent M, Preusser M, et al. EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood. *Nat Rev Clin Oncol.* 2021;18(3):170–186.
4. Fernandes C, Costa A, Osório L, et al. Glioblastoma. Chapter 11: current standards of care in glioblastoma therapy. *Codon Publications, Brisbane, Australia.* 2017.
5. Weller M, Cloughesy T, Perry JR, Wick W. Standards of care for treatment of recurrent glioblastoma--are we there yet? *Neuro Oncol.* 2013;15(1):4–27.
6. van Linde ME, Brahm CG, de Witt Hamer PC, et al. Treatment outcome of patients with recurrent glioblastoma multiforme: a retrospective multicenter analysis. *J Neurooncol.* 2017;135(1):183–192.
7. Wong ET, Hess KR, Gleason MJ, et al. Outcomes and prognostic factors in recurrent glioma patients enrolled onto phase II clinical trials. *J Clin Oncol.* 1999;17(8):2572–2578.
8. Taylor OG, Brzozowski JS, Skelding KA. Glioblastoma multiforme: an overview of emerging therapeutic targets. *Front Oncol.* 2019;9:963.
9. Pardridge WM. Drug transport across the blood–brain barrier. *J Cerebral Blood Flow Metabolism.* 2012;32(11):1959–1972.
10. Sweeney MD, Zhao Z, Montagne A, Nelson AR, Zlokovic BV. Blood-brain barrier: from physiology to disease and back. *Physiol Rev.* 2019;99(1):21–78.
11. Sarkaria JN, Hu LS, Parney IF, et al. Is the blood–brain barrier really disrupted in all glioblastomas? A critical assessment of existing clinical data. *Neuro Oncol.* 2017;20(2):184–191.
12. Doroshow DB, Eder JP, LoRusso PM. BET inhibitors: a novel epigenetic approach. *Ann Oncol.* 2017;28(8):1776–1787.
13. Shi J, Vakoc CR. The mechanisms behind the therapeutic activity of BET bromodomain inhibition. *Mol Cell.* 2014;54(5):728–736.

14. Filippakopoulos P, Qi J, Picaud S, et al. Selective inhibition of BET bromodomains. *Nature.* 2010;468(7327):1067–1073.

15. Wu X, Liu D, Gao X, et al. Inhibition of BRD4 suppresses cell proliferation and induces apoptosis in renal cell carcinoma. *Cell Physiol Biochem.* 2017;41(5):1947–1956.

16. Tan Y, Wang L, Du Y, et al. Inhibition of BRD4 suppresses tumor growth in prostate cancer via the enhancement of FOXO1 expression. *Int J Oncol.* 2018;53(6):2503–2517.

17. Shafran JS, Andrieu GP, Györffy B, Denis GV. BRD4 regulates metastatic potential of castration-resistant prostate cancer through AHNAK. *Mol Cancer Res.* 2019;17(8):1627–1638.

18. Yang H, Wei L, Xun Y, Yang A, You H. BRD4: an emerging prospective therapeutic target in glioma. *Mol Ther Oncolytics.* 2021;21:1–14.

19. Jermakowicz AM, Rybin MJ, Suter RK, et al. The novel BET inhibitor UM-002 reduces glioblastoma cell proliferation and invasion. *Sci Rep.* 2021;11(1):23370.

20. Berenguer-Daizé C, Astorgues-Xerri L, Odore E, et al. OTX015 (MK-8628), a novel BET inhibitor, displays in vitro and in vivo antitumor effects alone and in combination with conventional therapies in glioblastoma models. *Int J Cancer.* 2016;139(9):2047–2055.

21. Matzuk MM, McKeown MR, Filippakopoulos P, et al. Small-molecule inhibition of BRDT for male contraception. *Cell.* 2012;150(4):673–684.

22. Hottinger AF, Sanson M, Moyal E, et al. Dose optimization of MK-8628 (OTX015), a small molecule inhibitor of bromodomain and extra-terminal (BET) proteins, in patients (pts) with recurrent glioblastoma (GB). *J Clin Oncol.* 2016;34(suppl 15):e14123–e14123.

23. Moreno V, Sepulveda JM, Vieito M, et al. Phase I study of CC-90010, a reversible, oral BET inhibitor in patients with advanced solid tumors and relapsed/refractory non-Hodgkin's lymphoma. *Ann Oncol.* 2020;31(6):780–788.

24. Moreno V, Vieito Villar M, Sepulveda Sanchez JM, et al. CC-90010, a reversible, potent oral bromodomain and extraterminal inhibitor in patients with advanced solid tumors and relapsed/refractory diffuse large B cell lymphoma: longer follow-up from parts A & B and first reporting of part C of a phase 1 study. *Ann Oncol.* 2021;32(suppl 1):Abstract 8MO.

25. US Department of Health and Human Services, National Institutes of Health, National Cancer Institute. (2017) Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. Available at: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf. Accessed June 22, 2021.

26. Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: response assessment in Neuro-Oncology Working Group. *J Clin Oncol.* 2010;28(11):1963–1972.

27. van den Bent MJ, Wefel JS, Schiff D, et al. Response assessment in neuro-oncology (a report of the RANO group): assessment of outcome in trials of diffuse low-grade gliomas. *Lancet Oncol.* 2011;12(6):583–593.

28. Riccardi K, Li Z, Brown JA, et al. Determination of Unbound Partition Coefficient and in Vitro–in Vivo Extrapolation for SLC13A Transporter–Mediated Uptake. *Drug Metabolism and Disposition.* 2016;44(10):1633-1642.

29. Tsujikawa L, Norek K, Calosing C, et al. Preclinical development and clinical validation of a whole blood pharmacodynamic marker assay for the BET bromodomain inhibitor ZEN-3694 in metastatic castration-resistant prostate cancer (mCRPC) patients. *Cancer Res.* 2017;77(13 Supplement):Abstract LB-038.

30. Lin X, Huang X, Uziel T, et al. HEXIM1 as a robust pharmacodynamic marker for monitoring target engagement of BET family bromodomain inhibitors in tumors and surrogate tissues. *Mol Cancer Ther.* 2017;16(2):388–396.

31. Gallego O. Nonsurgical treatment of recurrent glioblastoma. *Curr Oncol.* 2015;22(4):e273–e281.

32. Merck & Co Inc. (2015) TEMODAR® (temozolomide) [package insert]. Available at: https://www.accessdata.fda.gov/drugsatfda_docs/label/2016/021029s031lbl.pdf. Accessed December 1, 2021.

33. Merck Sharp & Dohme B.V. (2022) TEMODAL® (temozolomide) [Summary of Product Characteristics]. Available at: https://www.ema.europa.eu/en/medicines/human/EPAR/temodal. Accessed March 04, 2022.

34. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med.* 2005;352(10):987–996.

35. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *Lancet Oncol.* 2009;10(5):459–466.

36. Abramson JS, Blum KA, Flinn IW, et al. BET inhibitor CPI-0610 is well tolerated and induces responses in diffuse large B-cell lymphoma and follicular lymphoma: preliminary analysis of an ongoing phase 1 study. *Blood.* 2015;126(23):1491.

37. Vieito M, Simonelli M, de Vos F, et al. Trotabresib (CC90010) in combination with adjuvant temozolomide or concomitant temozolomide plus radiotherapy in patients with newly diagnosed glioblastoma. *Neuro-Oncology Advances.* 2022;4(1).

38. Vieito M, Sepulveda JM, Moreno V, et al. Trotabresib (CC-90010) in combination with concomitant temozolomide plus radiotherapy and adjuvant temozolomide in patients with newly diagnosed glioblastoma: updated results from a phase 1b/2 study. *Neuro-Oncology.* 2022;24(Suppl 7):Abstract CTNI-21.

Figure legends

Figure 1. Trotabresib plasma PK [a]Patients were excluded if missing plasma concentration data led to AUC extrapolation > 25% and an adjusted R^2 value < 0.8. Abbreviations: AI, accumulation index; AUC_{24}, area under the trotabresib concentration–time curve from 0 to 24 hours; AUC_{inf}, area under the trotabresib concentration–time curve from 0 to infinity; C_{max}, peak trotabresib concentration; D, day; GCV, geometric coefficient of variation; GM, geometric mean; PK, pharmacokinetics; SD, standard deviation; $t_{½}$, trotabresib half-life; t_{max}, time to peak trotabresib concentration.

Figure 2. Trotabresib brain tumor tissue and CSF penetration. (A) individual patient concentrations and (B) summary statistics. Abbreviations: CSF, cerebrospinal fluid; GCV, geometric coefficient of variation; GM, geometric mean; SD, standard deviation. Blue data points indicate individual patient trotabresib concentrations. Black data points indicate median trotabresib concentrations.

Figure 3. Trotabresib pharmacodynamics: modulation of (A) mean ± SD *CCR1* and (B) individual patient *HEXIM1* mRNA in blood, (C) association between blood *HEXIM1* mRNA level modulation and plasma trotabresib concentration at the time of surgery, and (D) modulation of *HEXIM1* expression in brain tumor tissue. Abbreviation: SD, standard deviation.

Figure 4. TRAEs reported in ≥ 10% of patients at any grade or at grade 3/4 severity in ≥ 1 patient during the overall study by treatment period. (A) TRAEs reported from initiation of preoperative trotabresib 30 mg/day on days 1–4 until initiation of postoperative maintenance therapy (n = 20), and (B) TRAEs reported after initiation of postoperative maintenance trotabresib 45 mg/day 4 days on/24 days off (n = 16).

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; TRAE, treatment-related adverse event.

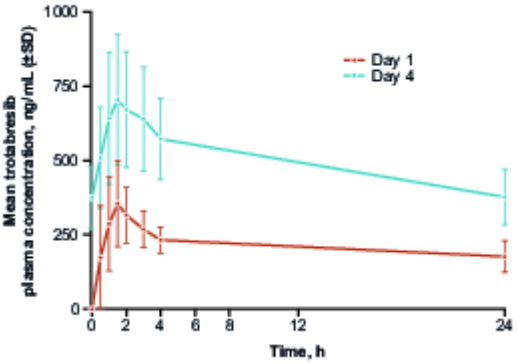
Figure 5. Treatment duration as of November 1, 2022. Abbreviations: M, methylated; Mut, mutant; NA, not available; NOS, not otherwise specified; U, unmethylated; WT, wild-type. Dotted line indicates approximate time of surgery.

Table 1. Patient characteristics. [a]*MGMT* promoter methylation status and *IDH* mutation status were determined using archival surgically resected tumor tissue and assessed per standard methodology at each institution. Abbreviations: ECOG PS, Eastern Cooperative Oncology Group performance status IDH, isocitrate dehydrogenase.

Patient characteristics	**N = 20**
Median age, years (range)	47 (33–75)
Sex, n (%)	
Male	14 (70)
Female	6 (30)
ECOG PS, n (%)	
0	12 (60)
1	8 (40)
Tumor type, n (%)	
Glioblastoma	19 (95)
Diffuse astrocytoma	1 (5)
MGMT promoter methylation status, n (%)[a]	
Methylated	7 (35)
Unmethylated	7 (35)
Not reported	6 (30)
IDH mutation status, n (%)[a]	
Wild-type	14 (70)
Mutant	5 (25)
Not otherwise specified	1 (5)
Number of prior radiation therapies, n (%)	
1	19 (95)
2	1 (5)
Number of prior systemic therapies, n (%)	
1	4 (20)
2	15 (75)
> 2	1 (5)
Median time since initial diagnosis, years (range)	1.2 (0.6–10.8)

Figure 1

A

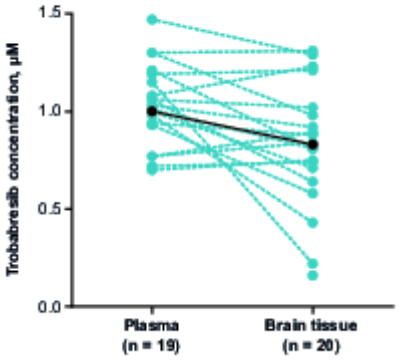


B

Plasma PK parameters, GM (% GCV)	Trotabresib 30 mg/day (n = 20)	
	Day 1	Day 4
C_{max}, ng/mL	392 (38)	720 (31)
Median t_{max}, h (range)	1.5 (0.5–23)	1.9 (0.9–3)
AUC_{24}, ng·h/mL[a]	5083 (15)	11250 (34)
Mean $t_{1/2}$, h (SD)[b]		46 (17)
AUC_{inf}, ng·h/mL[a]		34857 (47)
AI C_{max}	1.84	
AI AUC_{24}	2.21	

Figure 2

A



B

Parameter	GM (% GCV)
Plasma concentration, µM (n = 19)	1.02 (20%)
Brain tissue concentration, µM (n = 20)	0.74 (39%)
Mean tissue:plasma ratio (SD)	0.84 (± 0.25)
Tissue binding-corrected brain concentration, µM[a] (n = 20)	0.028 (39%)
CSF concentration, µM (n = 13)	0.14 (78%)
Mean CSF:plasma ratio (SD)	0.17 (± 0.64)

Figure 3

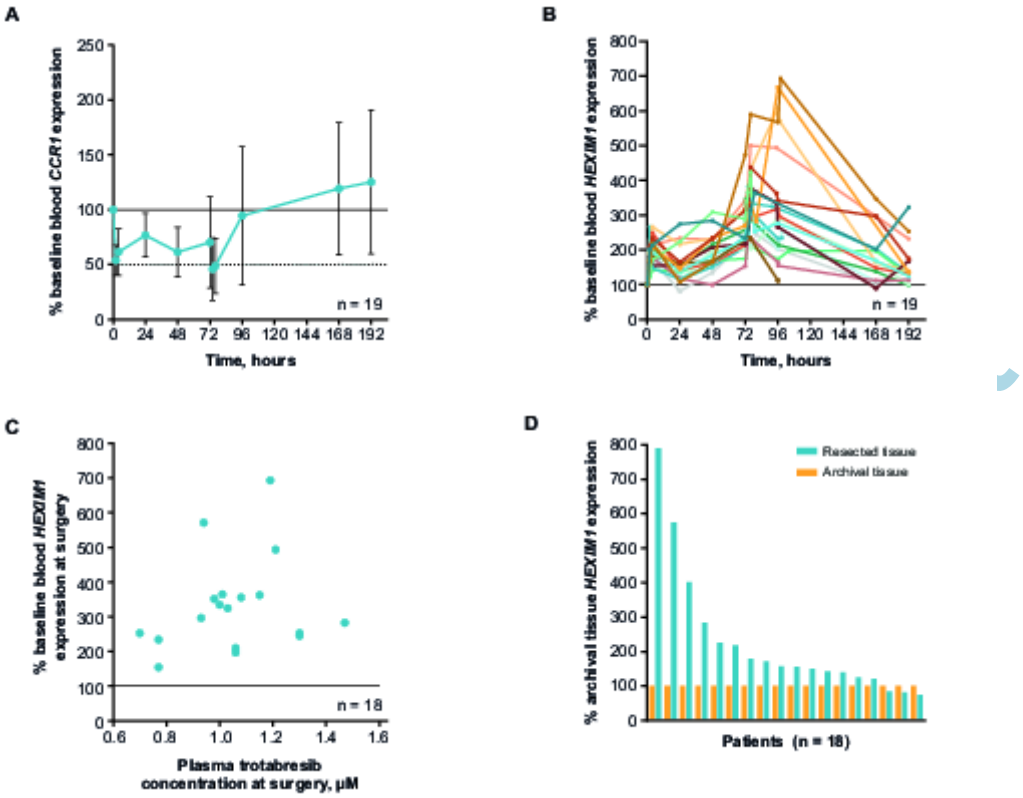


Figure 4

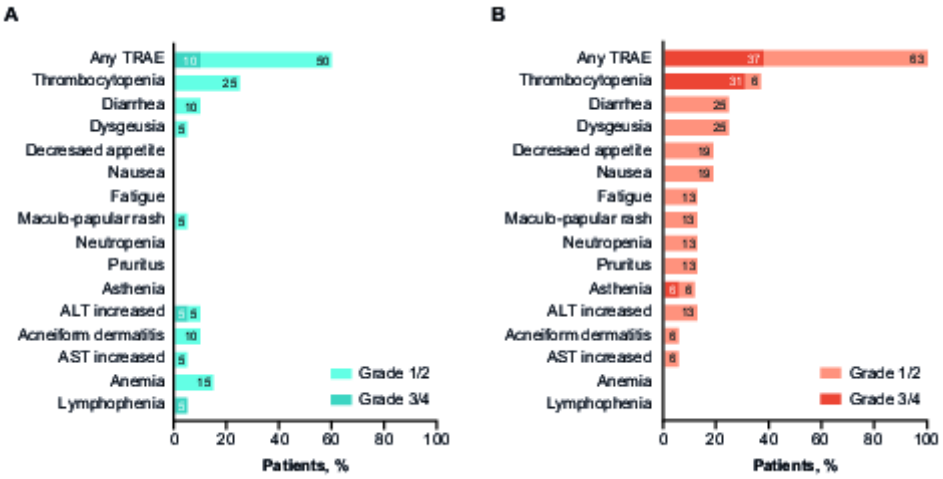


Figure 5