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ORIGINAL REPORT

Multicenter Phase IB Trial of Carboxyamidotriazole Orotate and Temozolomide for Recurrent and Newly Diagnosed Glioblastoma and Other Anaplastic Gliomas

Antonio Omuro, Kathryn Beal, Katharine McNeill, Robert J. Young, Alissa Thomas, Xuling Lin, Robert Terziev, Thomas J. Kaley, Lisa M. DeAngelis, Mariza Daras, Igor T. Gavrilovic, Ingo Mellinghoff, Eli L. Diamond, Andrew McKeown, Malbora Manne, Andrew Caterfino, Krishna Patel, Linda Bavisotto, Greg Gorman, Michael Lamson, Philip Gutin, Viviane Tabar, Debyani Chakravarty, Timothy A. Chan, Cameron W. Brennan, Elizabeth Garrett-Mayer, Rashida A. Karmali, and Elena Pentsova

Author affiliations and support information (if applicable) appear at the end of this article.

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Corresponding author: Elena Pentsova, MD, Department of Neurology, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, Box 52, New York, NY 10065; e-mail: pentsove@mskcc.org.

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ABSTRACT

Purpose

Carboxyamidotriazole orotate (CTO) is a novel oral inhibitor of non–voltage-dependent calcium channels with modulatory effects in multiple cell-signaling pathways and synergistic effects with temozolomide (TMZ) in glioblastoma (GBM) models. We conducted a phase IB study combining CTO with two standard TMZ schedules in GBM.

Methods

In cohort 1, patients with recurrent anaplastic gliomas or GBM received escalating doses of CTO (219 to 812.5 mg/m^2 once daily or 600 mg fixed once-daily dose) combined with TMZ (150 mg/m^2 5 days during each 28-day cycle). In cohort 2, patients with newly diagnosed GBM received escalating doses of CTO (219 to 481 mg/m^2/d once daily) with radiotherapy and TMZ 75 mg/m^2/d, followed by TMZ 150 mg to 200 mg/m^2 5 days during each 28-day cycle.

Results

Forty-seven patients were enrolled. Treatment was well tolerated; toxicities included fatigue, constipation, nausea, and hypophosphatemia. Pharmacokinetics showed that CTO did not alter TMZ levels; therapeutic concentrations were achieved in tumor and brain. No dose-limiting toxicities were observed; the recommended phase II dose was 600 mg/d flat dose. Signals of activity in cohort 1 (n = 27) included partial (n = 6) and complete (n = 1) response, including in O^6-methylguanine–DNA methyltransferase unmethylated and bevacizumab-refractory tumors. In cohort 2 (n = 15), median progression-free survival was 15 months and median overall survival was not reached (median follow-up, 28 months; 2-year overall survival, 62%). Gene sequencing disclosed a high rate of responses among *EGFR*-amplified tumors (P = .005), with mechanisms of acquired resistance possibly involving mutations in mismatch-repair genes and/or downstream components *TSC2*, *NF1*, *NF2*, *PTEN,* and *PIK3CA*.

Conclusion

CTO can be combined safely with TMZ or chemoradiation in GBM and anaplastic gliomas, displaying favorable brain penetration and promising signals of activity in this difficult-to-treat population.

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INTRODUCTION

The prognosis of patients with glioblastoma (GBM) and anaplastic gliomas (AG) is dismal. In newly diagnosed GBM, radiotherapy with concomitant and adjuvant temozolomide (TMZ) remains a mainstay of treatment, achieving a median overall survival (OS) of 16 to 18 months.[1-6] After treatment, virtually all patients recur, and the most common salvage therapy is bevacizumab, which achieves median OS of 6 to 9 months.[7-9] After bevacizumab failure, death ensues rapidly after a median OS of 2 to 4 months.[10,11]

Targeted therapies, including inhibitors of epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor, vascular endothelial growth factor (VEGF), mammalian target of rapamycin, phosphatidylinositol 3-kinase, and others have failed to improve survival in gliomas.[12]

ASSOCIATED CONTENT

Data Supplement
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As an alternative to targeting single pathway components, we have focused on modulation of calcium signaling to simultaneously block multiple Ca^{2+}-dependent pathways.[13] Intracellular Ca^{2+} homeostasis is markedly altered in cancer cells through increased expression or abnormal activation of Ca^{2+} channels, transporters, and Ca^{2+}-ATPases.[14,15] Free Ca^{2+} is a crucial component in multiple oncogenic processes, including cancer cell motility, invasion, angiogenesis, DNA damage response, transcription, telomerase function, differentiation, cell cycle, apoptosis, and immune evasion.[16]

Carboxyamidotriazole orotate (CTO), the orotate salt of 5-amino-1-(4-(4-chlorobenzoyl)-3,5-dichlorobenzyl)-1,2,3-triazole-4-carboxamide (CAI), is a novel oral inhibitor of non–voltage-dependent calcium channels under development for cancer therapy. Preclinical studies of single-agent CTO and CAI have demonstrated cytostatic effects deriving from the modulation of multiple signal-transduction pathways, including growth factors/phosphatidylinositol 3-kinase/Akt/PTEN/mammalian target of rapamycin, RAS/RAF/mitogen-activated protein kinase/MEK, Wnt-β-catenin, histone deacetylases (HDAC), heat shock protein 90, Bcr-Abl, and others.[13,17-23] Additional effects include reversal of tumor microenvironment immunosuppression through tumor necrosis factor-α inhibition in tumor-associated macrophages,[24] and metabolic effects through inhibition of cancer cell oxidative phosphorylation.[25] Importantly, CTO and CAI are capable of penetrating the blood-brain barrier (BBB) and accumulating in brain tissue, as suggested by studies of CTO in U251 GBM xenograft models that showed high tumor concentrations and marked synergistic activity in combination with TMZ.[22] Initial attempts to develop CAI and micronized CAI for clinical use were hampered by substantial systemic and neurologic toxicity, a variable pharmacokinetic (PK) profile, and poor bioavailability.[19,26,27] Conversely, CTO displays improved bioavailability and follows an optimized manufacturing process that decreased toxicity in comparison with CAI.[28] A first-in-human phase I study of single-agent CTO in advanced solid tumors showed that doses ranging from 75 to 427 mg/m^2/d were associated with a safe toxicity profile and predictable PK, although patients with brain tumors were excluded.[29] On the basis of our preclinical data, we conducted a phase IB study exploring the combination of CTO and different TMZ schedules in gliomas.

METHODS

The study was divided into two cohorts. Cohort 1 explored escalating doses of CTO combined with a standard TMZ schedule (150 mg/m^2/d, 5 days during each 28-day cycle)[30] in TMZ-refractory recurrent GBM and AG. After signals of activity were observed, a subsequent cohort (cohort 2) explored the incorporation of CTO into the standard TMZ chemoradiotherapy for newly diagnosed GBM.[1]

Dose-limiting toxicity (DLT) was defined as any of the following adverse events (AEs) occurring in the first cycle of treatment and was considered to be possibly, probably, or definitely related to study treatment (Data Supplement). A standard 3+3 dose-escalation phase I design was used (Data Supplement).

Primary objectives were determining safety, toxicity, maximum tolerated dose (MTD), and recommended phase II dose (RP2D) of CTO in combination with TMZ within respective lines of treatment. The secondary objective was PK evaluation. Exploratory objectives included evaluation of CTO brain intratumoral and CSF concentrations, preliminary evaluation of efficacy, and tumor genomic characterization. The protocol and informed consent were approved by the institutional review board at each institution. Written informed consent was obtained from all patients.

Eligibility Criteria

Cohort 1 enrolled patients with recurrent or progressive GBM (WHO IV) or grade 3 AGs, irrespective of previous bevacizumab exposure, number of previous treatments, or leptomeningeal spread. Cohort 2 enrolled patients with newly diagnosed GBM (Data Supplement).

Treatment and Evaluations

In cohort 1, TMZ 150 mg/m^2/d was given on days 1 to 5 of a 28-day cycle, combined with body surface area (BSA)-adjusted once-daily CTO (Table 1). Six additional patients with BSA 1.8 to 2 m^2 received a fixed flat 600 mg once-daily dose for additional PK evaluation.

In cohort 2, BSA-adjusted daily CTO was combined with TMZ 75 mg/m^2/d and standard focal radiotherapy (60 Gy in 2 Gy/d fractions through intensity-modulated radiation therapy or three-dimensional conformal external beam radiation therapy). After radiotherapy, CTO was continued and TMZ discontinued for 4 weeks, then resumed in 28-day adjuvant cycles at 150 mg/m^2/d on days 1 to 5 (cycle 1), and 200 mg/m^2/d on days 1 to 5 (cycle 2 and onwards, if well tolerated).

A maximum of 12 total (cohort 1) or adjuvant (cohort 2) TMZ cycles was allowed; single-agent CTO could be continued until progression. Radiographic responses were assessed by Macdonald Criteria, then revised using Response Assessment in Neuro-oncology (RANO) criteria.[31] PK evaluations are described in the Data Supplement. The current analysis extends through February 10, 2017.

Gene Sequencing

Available tumor samples were analyzed through massively parallel next-generation sequencing (NGS) using the Memorial Sloan Kettering–Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) assay.[32] In brief, MSK-IMPACT is a hybridization capture-based sequencing assay that provides full exon coverage of 410 cancer-related genes, detecting base substitutions, small indels, copy number alterations, and select gene rearrangements. The current analysis is restricted to mutations annotated as potentially oncogenic or in hotspot locations according to the OncoKB library.[33]

Statistical Considerations

OS was defined as the time from first treatment until death as a result of any cause. Progression-free survival (PFS) was defined as the time from first treatment until progression of disease or death as a result of any cause. For patients who did not have recorded progression or death, dates were censored at the last date the patient was known to be progression free and/or alive. Kaplan-Meier methods were used to estimate survival distributions.

Table 1. Distribution of Treated Patients (n = 42)

Dose Level (mg/m^2)	Cohort 1 Recurrent Disease	Cohort 2 Newly Diagnosed Disease
219	3	3
285	3	3
370	3	6
481	3	3
625	3	—
812.5	6	—
600*	6	—
Total	27	15

*Milligrams fixed once-daily dose.

RESULTS

Patient Characteristics and Dose Level Cohorts

A total of 47 patients were registered; 42 met the inclusion criteria and were treated in the study (n = 27 in cohort 1 and n = 15 in cohort 2; Table 1). Patient characteristics, *MGMT* promoter, and isocitrate dehydrogenase (IDH)-1 status are summarized in Table 2.

In cohort 1, patients were heavily pretreated, with 14 (52%) having experienced two or more recurrences. Two patients had tumor leptomeningeal spread. In addition to radiotherapy and various cytotoxic agents, previous treatments included anti-VEGF therapy (n = 4), other targeted therapy (n = 4), and immunotherapy (n = 1).

In cohort 2, all but one patient had a GBM; the one patient with a WHO grade 3 anaplastic astrocytoma had an *IDH1/2* wild-type and genomic signature consistent with GBM.

Table 2. Patient Characteristics

Characteristic	Cohort 1 (n = 27)	Cohort 2 (n = 15)
Age, years, median (range)	49 (28-78)	57.5 (24-77)
Sex		
Male	19 (70)	7 (47)
Female	8 (30)	8 (53)
Ethnicity		
White	24 (88)	13 (86)
Asian	1 (4)	1 (7)
Black or African American	1 (4)	1 (7)
Hispanic or Latino	—	—
N/A	1 (4)	—
ECOG performance status		
0	13 (48)	9 (60)
1	11 (41)	5 (33)
2	3 (11)	1 (7)
No. of previous recurrence(s)		
1	13 (48)	NA
2	9 (33)	NA
≥ 3 (3-8)	5 (19)	NA
Leptomeningeal disease	2 (7)	—
Previous systemic treatment		
Temozolomide	25 (93)	NA
Other cytotoxic agents	6 (22)	NA
Bevacizumab or anti-VEGF treatment	4 (15)	NA
Targeted therapy	4 (15)	NA
Immunotherapy	1 (4)	NA
Tumor treating fields	1 (4)	NA
Histology at diagnosis		
Glioblastoma WHO IV	16 (59)	14 (93)
Gliosarcoma WHO IV	1 (4)	—
Anaplastic astrocytoma WHO III	8 (30)	1 (7)
Anaplastic oligodendroglioma WHO III	1 (4)	—
Anaplastic glioma NOS WHO III	1 (4)	—
IDH-1 or IDH-2 status		
Mutant	8 (30)	2 (13)
Wild type	13 (48)*	9 (60)
Not tested	6 (22)	4 (27)
MGMT promoter		
Methylated	9 (33)	4 (27)
Unmethylated	11 (41)	7 (47)
Not performed or equivocal	7 (26)	4 (27)

NOTE. Data are presented as No. (%) unless indicated otherwise. "—" indicates zero. Collected safety data included adverse events, vital signs, ECG, clinical laboratory tests, and physical examinations. Toxicities were reported according to National Cancer Institute Common Terminology Criteria for Adverse Events (version 4.03).

Abbreviations: ECOG, Eastern Cooperative Oncology Group; IDH, isocitrate dehydrogenase; MGMT, O^6-methylguanine–DNA methyltransferase; N/A, not available; NA, not applicable; NOS, not otherwise specified; VEGF, vascular endothelial growth factor.

*Three of these patients were only tested for IDH-1 R132H by immunohistochemistry.

Toxicity

Cohort 1. The treatment was well tolerated, with no DLTs observed at any dose level. The most common treatment-related AEs were fatigue, nausea, and constipation and hypophosphatemia (Table 3). No grade 3 to 5 treatment-related AEs occurred. Two patients in the 481 mg/m^2 cohort discontinued treatment because of headache and left-sided weakness; neither was considered treatment related. There were no significant ECG QTc abnormalities. Four patients had a grade 1 or 2 reversible blurry vision. Despite the absence of DLTs, a decision was made not to pursue additional escalation, given that high dose levels had been explored and PK analysis had shown therapeutic levels in plasma and tumor, with no additional increments in drug exposure with increased doses. An MTD was therefore not reached, and the dose of CTO 812 mg/m^2/d was the maximum administered dose.

Cohort 2. Treatment was well tolerated but toxicities were more frequent than in cohort 1, in line with the higher TMZ dose intensity and concomitant radiotherapy. The most common AEs were fatigue, nausea, constipation, radiotherapy-related dermatitis, thrombocytopenia, skin rash, and headache (Table 4). At doses up to 481 mg/m^2, no DLTs were observed during the DLT evaluation period. However, late toxicities developed beyond the DLT observation: grade 3 febrile neutropenia (n = 2), grade 3 neutropenia (n = 1), grade 4 platelets (n = 1), and grade 3 ALT (n = 1). After completion of the 481 mg/m^2 cohort, PK results became available, and dose escalation was halted, with 481 mg/m^2 declared the maximum administered dose. The next lower dose level (370 mg/m^2) was expanded to a total of six patients, and no DLTs were observed.

PK and Determination of Recommended Phase II Dose

PK results are summarized in the Data Supplement. In cohort 1, maximum time to reach maximum concentration ranged from 4 to 8 hours after the dose. Thereafter, the concentration profile of CAI remained flat over 24 hours. In general, interpatient variability was observed, and CAI exposure did not exhibit an expected dose-response with increasing doses of CTO, contrary to previously observed results in single-agent studies.[29] Drug exposure plateaued at doses of 370 mg/m^2; therefore, assuming an average BSA, additional testing of a flat fixed dose of 600 mg daily was performed in an expansion cohort of six patients, which displayed similar PK parameters. The fixed 600 mg daily dose was declared the RP2D for cohort 1. Similarly, but independently for cohort 2, the RP2D declared was 370 mg/m^2 (this cohort was expanded to six patients after de-escalation from the 481 mg/m^2 dose level). The PK justification for RP2D of 600 mg once daily for cohorts 1 and 2 is provided in the Data Supplement.

Analysis of CTO and TMZ interactions showed that CAI concentrations at day 5 were approximately 50% lower than at day 29, suggesting that TMZ likely influences CAI levels. In contrast,

Table 3. Cohort 1 AEs Possibly, Probably, or Definitely Related to CTO and TMZ Reported by ≥ 10% of Patients

AE	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Overall
General disorders and administrative site conditions						
Fatigue	7 (25.9)	7 (25.9)	0 (0.0)	0 (0.0)	0 (0.0)	14 (51.8)
GI disorders						
Nausea	11 (40.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	11 (40.7)
Constipation	10 (37.0)	1 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)	11 (40.7)
Vomiting	7 (25.9)					7 (25.9)
Skin and subcutaneous disorders						
Dry skin	3 (11.1)	1 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)	4 (14.8)
Metabolism and nutrition disorders						
Hypophosphatemia	1 (3.70)	2 (7.4)	0 (0.0)	0 (0.0)	0 (0.0)	3 (11.1)
Nervous system disorders						
Dizziness	3 (11.1)	1 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)	4 (14.8)
Eye disorders						
Blurred vision	3 (11.1)	1 (3.7)	0 (0.0)	0 (0.0)	0 (0.0)	4 (14.8)

NOTE. Data are presented as No. (%). Toxicity data were aggregated by counting the number of patients who had the AE, and counting the highest grade of a given AE across all cycles for all patients. AE causality was not broken down separately by drug treatment or different doses of CTO and TMZ.
Abbreviations: AE, adverse event; CTO, carboxyamidotriazole orotate; TMX, temozolomide.

TMZ levels were not significantly altered by CTO. PK analyses in cohort 2 corroborated the findings in cohort 1.

Tumor and CSF CAI Levels. Across both cohorts, six patients had tumors analyzed for CAI levels, and all displayed intratumoral drug concentrations (1,020 to 6,200 ng/g) above therapeutic levels, estimated at > 400 ng/mL, including samples obtained[19,20] from areas both with and without BBB disruption (Data Supplement). Concomitant CAI plasma concentrations ranged from 213 to 3,460 ng/mL, respectively, indicating a tissue accumulation effect. In an unplanned analysis, two CSF specimens collected approximately 6 hours after CTO dosing showed lower concentrations (22.1 and 31.9 ng/mL) than did corresponding plasma or tumor tissue.

Exploratory Evaluation of Activity and Efficacy

In cohort 1, all 27 patients had measurable disease per RANO criteria and were considered evaluable for response, including four patients unable to complete the first two cycles because of early progression. One achieved a complete response (CR), and six achieved a partial response (PR), for an objective response rate of 26% (95% CI, 11% to 46%; Fig 1). Another 11 patients (41%)

Table 4. Cohort 2 AEs Possibly, Probably, or Definitely Related to CTO and TMZ or Radiotherapy Reported by ≥ 10% of Patients

AE	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Overall
GI disorders						
Nausea	6 (40.0)	1 (6.7)	1 (6.7)	0 (0.0)	0 (0.0)	8 (53.3)
Constipation	4 (26.7)	3 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	7 (46.7)
General disorders and administrative site conditions						
Fatigue	4 (26.7)	6 (40.0)	0 (0.0)	0 (0.0)	0 (0.0)	10 (66.7)
Mucosal inflammation	2 (13.3)					2 (13.3)
Injury, poisoning and procedural						
Dermatitis radiation	4 (26.7)	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	5 (33.3)
Investigations						
Platelet decreased	1 (6.7)	1 (6.7)	1 (6.7)	1 (6.7)	0 (0.0)	4 (26.7)
ALT increase	0 (0.0)	2 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)	2 (13.3)
White blood cell count decreased	0 (0.0)	1 (6.7)	1 (6.7)	0 (0.0)	0 (0.0)	2 (13.3)
Neutrophil count decreased	0 (0.0)	1 (6.7)	0 (0.0)	1 (6.7)	0 (0.0)	2 (13.3)
Nervous system disorders						
Headache	3 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (20.0)
Hemiparesis	1 (6.7)	1 (6.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (13.3)
Dysgeusia	2 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (13.3)
Skin and subcutaneous tissue						
Rash NOS	1 (6.7)	2 (13.3)	1 (6.7)	0 (0.0)	0 (0.0)	4 (26.7)
Infections and infestations						
Thrush	2 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (13.3)
Blood and lymphatic system disorders						
Febrile neutropenia	0 (0.0)	0 (0.0)	2 (13.3)	0 (0.0)	0 (0.0)	2 (13.3)
Anemia	2 (13.3)	0 (0.0)	1 (6.7)	0 (0.0)	0 (0.0)	3 (20.0)

NOTE. Data are presented as No. (%). Toxicity data were aggregated by counting the number of patients who had the AE, and counting the highest grade of a given AE across all cycles for all patients. AE causality was not broken down separately by drug treatment, radiotherapy, or different doses of CTO and TMZ.
Abbreviations: AE, adverse event; CTO, carboxyamidotriazole orotate; TMZ, temozolomide.

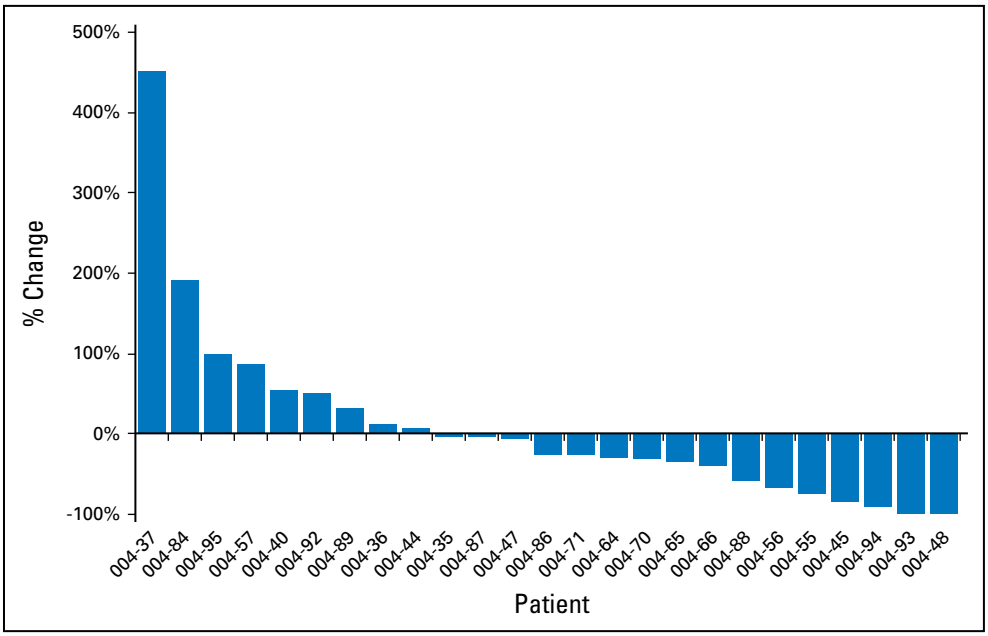

Fig 1. Waterfall plot showing best radiographic response as changes in sum of product of diameters. (*) Additional four patients progressed clinically before the first scan and are not shown.

had stable disease (SD); many of them displayed a reduction in tumor size under the response threshold, as shown in the waterfall plot (Fig 1). Nine patients (33%) had progression of disease (PD), for a clinical benefit (CR, PR, or SD for two cycles) rate of 67% (95% CI, 46% to 83%). Responses occurred at 285 to 481 mg/m^2 and at the 600 mg flat fixed once-daily dose and lasted up to 14 months. Responders (Data Supplement) included typically chemoresistant tumors such as four IDH wild-type, *MGMT* unmethylated tumors and two GBMs recurring after failure of anti-VEGF therapy. The median OS was 10.2 months (95% CI, 8 to 30 months) and 1-year OS was 46% (95% CI, 31% to 70%). The median PFS was 3.1 months (95% CI, 2.5 to 6.8 months) and 6-month PFS was 37% (95% CI, 12 to 62 months).

In cohort 2 (n = 15), the median PFS was 15 months (95% CI, 5 to infinity), 1-year PFS was 52% (95% CI, 23% to 75%), and 2-year PFS was 35% (95% CI, 11% to 61%). The median OS was not reached after median follow-up of survivors of 28 months (Fig 2). The 1-year OS was 93% (95% CI, 61% to 99%), and 2-year OS was 62% (95% CI, 31% to 82%). Within this cohort, five patients were considered nonevaluable for response: four had no measurable disease and one withdrew consent. Nine patients with measurable disease by RANO criteria were evaluable for response evaluation, of whom one achieved a CR (Fig 2), two had a PR, three had SD, and three had PD. Two of the patients with PD underwent surgical resection, and prominent treatment effects were observed. Because minimal residual tumor was also seen, their response assessment remained PD. However, one of these patients was kept on study, resumed treatment, and remained stable and alive at $\geq$ 29 months; the other was removed from the study but survived for 20 months.

Gene Sequencing in Responders and Progressors

The results of NGS, as well as other available molecular information, are shown in the Data Supplement. Analysis was mostly exploratory and descriptive and was restricted to samples obtained in surgeries for routine clinical care, typically at diagnosis, or at recurrences requiring tumor debulking.

Responses were observed within various tumor phenotypes and presumed oncogenic drivers. The population of responders was enriched for amplification or hotspot mutations in *EGFR* (P = .005). Three of six responders with known *EGFR* status in cohort 1, and all three responders in cohort 2, had an *EGFR* amplification or oncogenic mutation. Evidence of clinical benefit was also observed in the other two remaining patients with *EGFR* amplification: one (patient 004-89) had a minor response or SD for 6 months in cohort 1; the other (patient 004-82), a cohort 2 patient, was nonevaluable for response because of the timing of the baseline magnetic resonance imaging but was stable for 24 months. None of the nine other nonresponders in cohort 1 had *EGFR* amplification. There were no correlations between response and IDH-1 or O^6-methylguanine–DNA methyltransferase (MGMT) status.

In tissue obtained at recurrence, four previous responders in cohort 1 and one in cohort 2 displayed a hypermutator phenotype,[34] here defined as $>$ 30 nonsynonymous point or indel mutations. This phenotype was also observed at baseline in one of the nonresponders in cohort 1 but in no other nonresponder either at baseline or at recurrence.

Analysis of oncogenic or hotspot mutations in both hypermutator and nonhypermutator tumors at recurrence disclosed emerging clones harboring *TSC2* mutations in four previous responders. A variety of mutations affecting downstream signaling pathway components and other genes was also observed after treatment exposure, including mutations in *PTEN*, *NF1*, *NF2*, *PIK3CA*, *H3F3A*, and others.

DISCUSSION

In this phase IB study, patients with recurrent (cohort 1) and newly diagnosed (cohort 2) GBM and other AG received escalating doses of CTO, a novel oral inhibitor of non–voltage-dependent calcium

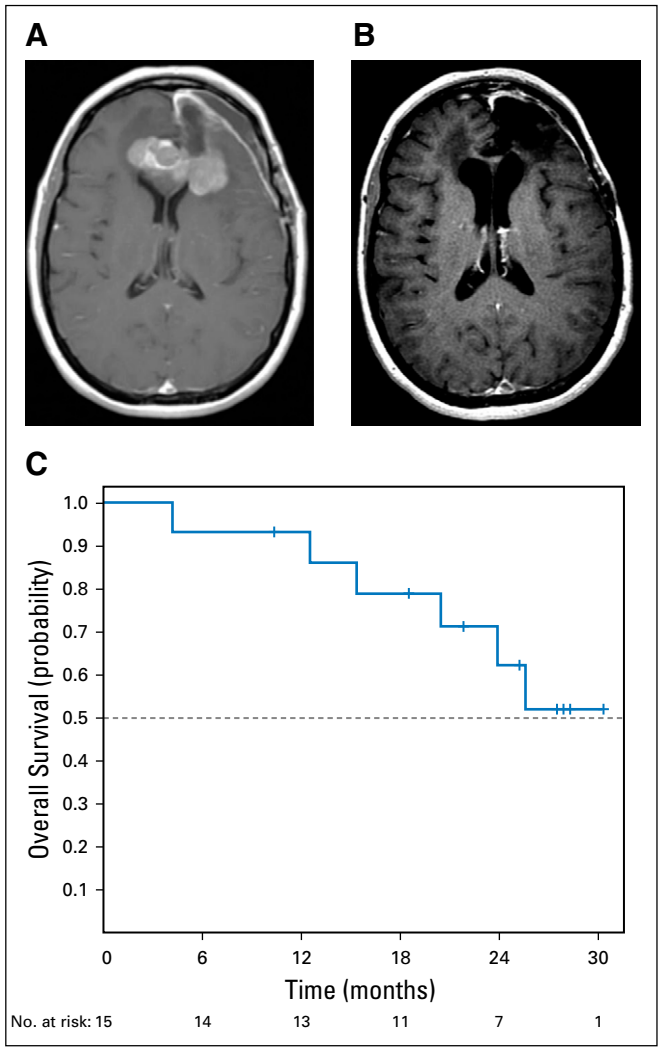

Fig 2. Cohort 2: example of durable radiographic complete response and Kaplan-Meier overall survival (OS) curve. Axial T1 postcontrast magnetic resonance imaging (A) at baseline after surgery and (B) after 32 months of treatment, showing complete resolution of enhancing mass crossing the corpus callosum. (C) OS Kaplan-Meier curve for all patients enrolled in cohort 2 (n = 15).

channels, in combination with TMZ. The treatment was safe and well tolerated. An MTD was not reached and, on the basis of PK data, the RP2D CTO dose for both cohorts was a flat fixed 600 mg once-daily dose. Importantly, analysis of resected tumor specimens showed that despite wide interpatient PK variability, therapeutically meaningful tumor concentrations were achieved in the brain, confirming the favorable BBB penetration properties predicted by preclinical models.

Although not a primary objective of this study, and despite the phase I dose-escalation design, encouraging signs of activity were seen. In cohort 1, consisting of patients with refractory, heavily pretreated tumors, responses were observed across different dose levels, for a total of seven responding patients. Although some of these harbored *IDH-1* mutations, responses were also seen in notoriously difficult-to-treat tumors such as *IDH-1* wild-type, unmethylated *MGMT* GBMs, and tumors previously failing anti-VEGF therapy. Interestingly, there was a trend in the number of cycles necessary to achieve a response, with two to four cycles required in methylated MGMT tumors, and four to eight cycles in unmethylated MGMT tumors. In cohort 2, the observed median PFS of 15 months, 2-year OS of 62%, and median OS not reached after median follow-up of 28 months are all far superior to those of standard TMZ chemoradiotherapy, typically associated with PFS of 6 to 7 months and OS of 16 to 19 months.[3,27,35] Our results are at odds with a previous study of CAI combined with radiotherapy in GBM, which failed to improve survival and found moderate to severe toxicities in one third of patients with a median treatment duration of only 2 months.[2] This discrepancy may be explained by the better toxicity profile and bioavailability of CTO, and by the combination with a cytotoxic drug, which might be crucial for this class of agent. Of note, signals of activity resulting from calcium-signaling modulation and cytotoxic chemotherapy were also observed with the combination of mibefradil and TMZ.[15]

Targeting of calcium-dependent pathways is associated with multiple downstream effects, and the relevant mechanisms underlying sensitivity and resistance may differ among unique patients. Likewise, the mechanisms underlying the synergistic effects between CTO and TMZ remain to be elucidated and could involve inhibition of HDAC, Wnt-β catenin, and poly (ADP-ribose) polymerase inhibitor, and restoration of p53, together with modulation of multiple anti-apoptotic factors, including EGFR, MEK, RAS, heat shock protein 90, and calcium-related growth factors.[17,18,21,22,36,37] In an exploratory fashion, tissue samples were analyzed for the presence of potential oncogenic or hotspot mutations on NGS that could predict response and provide clues as to the most relevant targets susceptible to calcium blockade. Analysis revealed that a wide range of phenotypes responded to treatment, in keeping with the hypothesis that CTO may overcome chemoresistance and inhibit multiple signaling pathways. However, the population of responders seemed particularly enriched for tumors harboring an *EGFR* amplification, with all such patients seeming to benefit from treatment. Interestingly, analysis of post-treatment tumor specimens showed that a large proportion of patients (31%) displayed a hypermutator phenotype,[34] all of which were observed among responding patients. This phenotype is typically triggered by TMZ-induced mutations in mismatch-repair genes and has been described previously in recurrent glioma.[34] Tissue obtained after chemoradiotherapy and before study enrollment was not available in most cohort 1 patients; therefore, it is not possible to determine if the hypermutation status preceded study entry. At least one patient displayed a hypermutator phenotype before enrollment, and cell lines from a variety of tumors displaying mismatch-repair deficiencies have been shown to be selectively sensitive to calcium blockade,[38] which could potentially explain the high frequency of hypermutators among responders. However, at least one patient developed a hypermutator phenotype in cohort 2 after exposure to CTO and TMZ, and a more plausible explanation may be that the hypermutation status developed as a consequence of prolonged TMZ exposure in patients treated successfully. Additional analysis of oncogenic or hotspot mutations in both hypermutators and nonhypermutators after treatment failure disclosed emerging clones harboring *TSC2* mutations in four of the previous responders. Other mutations affecting downstream pathway components, including *PTEN*, *NF1*, *NF2,* and *PIK3CA* mutations, were also observed,[39] suggesting that combinations with additional agents targeting such pathway components may be of interest. On the basis of the favorable toxicity profile, brain penetration,

and encouraging activity observed in this difficult-to-treat population of patients with GBM and AG, randomized phase II trials with CTO in combination with TMZ or chemoradiation are planned.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

AUTHOR CONTRIBUTIONS

Conception and design: Antonio Omuro, Rashida A. Karmali
Administrative support: Andrew McKeown, Malbora Manne, Andrew Caterfino, Krishna Patel, Rashida A. Karmali
Provision of study materials or patients: Andrew Caterfino
Collection and assembly of data: Antonio Omuro, Kathryn Beal, Katharine McNeill, Robert J. Young, Alissa Thomas, Xuling Lin, Robert Terziev, Thomas J. Kaley, Lisa M. DeAngelis, Mariza Daras, Igor T. Gavrilovic, Ingo Mellinghoff, Eli L. Diamond, Andrew McKeown, Malbora Manne, Andrew Caterfino, Krishna Patel, Linda Bavisotto, Greg Gorman, Philip Gutin, Viviane Tabar, Debyani Chakravarty, Timothy A. Chan, Cameron W. Brennan, Rashida A. Karmali, Elena Pentsova
Data analysis and interpretation: Antonio Omuro, Robert J. Young, Alissa Thomas, Xuling Lin, Malbora Manne, Krishna Patel, Linda Bavisotto, Greg Gorman, Michael Lamson, Cameron W. Brennan, Elizabeth Garrett-Mayer, Rashida A. Karmali, Elena Pentsova
Manuscript writing: All authors
Final approval of manuscript: All authors
Accountable for all aspects of the work: All authors

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Affiliations

Antonio Omuro, University of Miami, Miami, FL; **Kathryn Beal**, **Robert J. Young**, **Thomas J. Kaley**, **Lisa M. DeAngelis**, **Mariza Daras**, **Igor T. Gavrilovic**, **Ingo Mellinghoff**, **Eli L. Diamond**, **Andrew McKeown**, **Malbora Manne**, **Andrew Caterfino**, **Krishna Patel**, **Philip Gutin**, **Viviane Tabar**, **Debyani Chakravarty**, **Timothy A. Chan**, **Cameron W. Brennan**, and **Elena Pentsova**, Memorial Sloan Kettering Cancer Center; **Rashida A. Karmali**, Tactical Therapeutics, Inc, New York; **Katharine McNeill**, Montefiore Medical Center, Bronx, NY; **Alissa Thomas**, University of Vermont, Burlington, VT; **Xuling Lin**, National Neuroscience Institute, Singapore; **Robert Terziev**, University Hospital, Zurich, Switzerland; **Linda Bavisotto**, Porta Clinica PLLC, Seattle, WA; **Greg Gorman**, Samford University McWhorter School of Pharmacy, Birmingham, AL; **Michael Lamson**, Nuventra Pharma Sciences, Durham, NC; and **Elizabeth Garrett-Mayer**, Medical University of South Carolina, Charleston, SC.

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Multicenter Phase IB Trial of Carboxyamidotriazole Orotate and Temozolomide for Recurrent and Newly Diagnosed Glioblastoma and Other Anaplastic Gliomas

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Antonio Omuro
Consulting or Advisory Role: Bristol-Myers Squibb, Merck, Stemline Therapeutics, Novocure, Inovio Pharmaceuticals, Alexion Pharmaceuticals, AstraZeneca

Kathryn Beal
Stock or Other Ownership: MMT, MMT (I)

Katharine McNeill
Employment: Siemens Healthcare Diagnostics (I)
Patents, Royalties, Other Intellectual Property: Machinery patents for a medication infusion system (I)

Robert J. Young
Stock or Other Ownership: Agios, Alexion Pharmaceuticals, Biogen Idec, Celgene, Gilead Sciences, Karyopharm Therapeutics, Spark Therapeutics, Regeneron Pharmaceuticals, Stemline Therapeutics, Vertex
Consulting or Advisory Role: Agios
Research Funding: Agios (Inst)

Alissa Thomas
No relationship to disclose

Xuling Lin
No relationship to disclose

Robert Terziev
No relationship to disclose

Thomas J. Kaley
No relationship to disclose

Lisa M. DeAngelis
Consulting or Advisory Role: Pharmaco-Kinesis, Genentech, Roche, Sapience Therapeutics, Juno Therapeutics

Mariza Daras
No relationship to disclose

Igor T. Gavrilovic
No relationship to disclose

Ingo Mellinghoff
No relationship to disclose

Eli L. Diamond
No relationship to disclose

Andrew McKeown
No relationship to disclose

Malbora Manne
No relationship to disclose

Andrew Caterfino
No relationship to disclose

Krishna Patel
No relationship to disclose

Linda Bavisotto
Consulting or Advisory Role: Tactical Therapeutics, Acerta Pharma, Merrimack Pharmaceuticals, Berg Pharma, Arbutus Biopharma

Greg Gorman
No relationship to disclose

Michael Lamson
Employment: Nuventra Pharma Sciences
Consulting or Advisory Role: Tactical Therapeutics

Philip Gutin
No relationship to disclose

Viviane Tabar
No relationship to disclose

Debyani Chakravarty
No relationship to disclose

Timothy A. Chan
Leadership: Cancer Genetics, Illumina, Bristol-Myers Squibb
Stock or Other Ownership: Gritstone Oncology
Research Funding: Bristol-Myers Squibb
Patents, Royalties, Other Intellectual Property: Neoantigen discovery and genomic biomarkers for immunotherapy response

Cameron W. Brennan
Stock or Other Ownership: AVEO Pharmaceuticals
Travel, Accommodations, Expenses: Genentech/Roche

Elizabeth Garrett-Mayer
Stock or Other Ownership: Abbott Laboratories, Abbvie
Consulting or Advisory Role: Tactical Therapeutics, Okava Pharmaceuticals

Rashida A. Karmali
Employment: Tactical Therapeutics
Leadership: Tactical Therapeutics
Stock or Other Ownership: Tactical Therapeutics
Patents, Royalties, Other Intellectual Property: Tactical Therapeutics
Travel, Accommodations, Expenses: Tactical Therapeutics

Elena Pentsova
Consulting or Advisory Role: AstraZeneca

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