

# First-in-Human Phase I Clinical Trial of Pharmacologic Ascorbate Combined with Radiation and Temozolomide for Newly Diagnosed Glioblastoma



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## Abstract

**Purpose:** Standard treatment for glioblastoma (GBM) includes surgery, radiation therapy (RT), and temozolomide (TMZ), yielding a median overall survival (OS) of approximately 14 months. Preclinical models suggest that pharmacologic ascorbate (P-AsCH<sup>-</sup>) enhances RT/TMZ antitumor effect in GBM. We evaluated the safety of adding P-AsCH<sup>-</sup> to standard RT/TMZ therapy.

**Patients and Methods:** This first-in-human trial was divided into an RT phase (concurrent RT/TMZ/P-AsCH<sup>-</sup>) and an adjuvant (ADJ) phase (post RT/TMZ/P-AsCH<sup>-</sup> phase). Eight P-AsCH<sup>-</sup> dose cohorts were evaluated in the RT phase until targeted plasma ascorbate levels were achieved ( $\geq 20$  mmol/L). In the ADJ phase, P-AsCH<sup>-</sup> doses were escalated in each subject at each cycle until plasma concentrations were  $\geq 20$  mmol/L. P-AsCH<sup>-</sup> was infused 3 times weekly during the

RT phase and 2 times weekly during the ADJ phase continuing for six cycles or until disease progression. Adverse events were quantified by CTCAE (v4.03).

**Results:** Eleven subjects were evaluable. No dose-limiting toxicities occurred. Observed toxicities were consistent with historical controls. Adverse events related to study drug were dry mouth and chills. Targeted ascorbate plasma levels of 20 mmol/L were achieved in the 87.5 g cohort; diminishing returns were realized in higher dose cohorts. Median progression-free survival (PFS) was 9.4 months and median OS was 18 months. In subjects with undetectable MGMT promoter methylation ( $n = 8$ ), median PFS was 10 months and median OS was 23 months.

**Conclusions:** P-AsCH<sup>-</sup>/RT/TMZ is safe with promising clinical outcomes warranting further investigation.

## Introduction

Glioblastoma (GBM) is the most common primary brain malignancy in the United States (1). Standard treatment for GBM includes a combination of maximum safe surgical resection, followed by radiotherapy (RT) and temozolomide (TMZ) fol-

lowed by additional cycles of TMZ (2, 3). Despite advances in GBM treatment, including the development of new biological agents, patients with GBM continue to have a dismal prognosis with a median OS of 14 to 16 months, progression-free survival (PFS) of approximately 7 months, and 5-year survival of <10% (2, 4, 5).

Pharmacologic ascorbate (P-AsCH<sup>-</sup>), defined as a plasma ascorbate level  $\geq 10$  mmol/L has reemerged as a possible cancer therapeutic being toxic to tumor cells yet relatively innocuous to normal cells (6–8). Recent phase I clinical trials demonstrate the safety of P-AsCH<sup>-</sup> when administered with concurrent gemcitabine for stage IV pancreatic cancer (9), when combined with paclitaxel and carboplatin for ovarian cancer (10), as well when combined with radiation and gemcitabine for locally advanced pancreatic cancer (11). *In vitro* and orthotopic animal models demonstrate that P-AsCH<sup>-</sup> selectively increases cancer cell oxidative stress, thereby sensitizing cancer cells to radiation and chemotherapy (6, 12, 13).

On the basis of both the preclinical and clinical data, we designed and conducted a first-in-human phase I trial in newly diagnosed GBM subjects to estimate the MTD of P-AsCH<sup>-</sup> when added to the RT/TMZ regimen described by Stupp and colleagues (2, 4). The primary objective was to determine the safety and acute toxicity of P-AsCH<sup>-</sup> when administered with standard

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**Note:** Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

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### Translational Relevance

Pharmacologic ascorbate (intravenous infusions of high-dose vitamin C) has been shown to be efficacious in preclinical studies, both *in vitro* and *in vivo*, for treatment of glioblastoma multiforme (GBM). This study translates the preclinical findings into a phase I, first-in-human clinical trial combining pharmacologic ascorbate with radiation and temozolomide in the treatment of GBM. The primary objective was to determine the safety of this combination and explore the impact, if any, on treatment outcome. Data support pharmacologic ascorbate is safe and well tolerated when combined with radiation and temozolomide and may improve therapeutic outcome. Clinical trials combining pharmacologic ascorbate, temozolomide, and radiation continue.

therapy for GBM and to identify a recommended dose for a phase II clinical trial with P-AsCH<sup>-</sup>. Secondary objectives were to estimate PFS and overall survival (OS) as well as characterize an initial safety profile. GBM was felt a promising site for P-AsCH<sup>-</sup> application because of its poor prognosis as well as the known high concentration of ascorbate in the cerebrospinal fluid and central nervous system tissues (13–15).

## Patients and Methods

### Ethics and oversight

This trial was designed by the principal investigator (J.M. Buatti) and supervising statisticians (M.J. TenNapel and B.J. Smith). The protocol was submitted to FDA under IND 105715 in 2012 (J.J. Cullen, sponsor) and registered on ClinicalTrials.gov 19 December, 2012 under NCT01752491. Approval was obtained from The University of Iowa Institutional Review Board (Biomedical IRB01; IRB 201211713). Informed written consent was obtained from each subject before beginning the trial. The trial was conducted according to the Declaration of Helsinki, the Belmont Report, the United States Common Rule (45CFR§46), and the International Council on Harmonisation—Good Clinical Practice (GCP); all investigators were GCP trained. The University of Iowa Hospitals and Clinics Data and Safety Monitoring Committee (DSMC) reviewed data for compliance to protocol and participant safety. Safety and annual reports about this trial were submitted as required (21CFR§312.23, §312.32).

### Design

This open-label, single-center investigator-initiated clinical trial was divided into two phases: an RT phase and an adjuvant (ADJ) phase. To identify the recommended phase II dose of P-AsCH<sup>-</sup> during the RT phase, Storer's two-phase design B/D was employed (16). Briefly, a single participant is assigned to a P-AsCH<sup>-</sup> dose cohort. If no dose-limiting toxicities (DLT) occur, a second participant is assigned to the next-dose cohort. After a DLT occurs, a traditional 3 + 3 dose evaluation is used for the remaining P-AsCH<sup>-</sup> dose cohorts (16). With the 3 + 3 dose evaluation, a dose level is deemed too toxic if 2 out of 3 participants experience DLTs. Thus, the recommended phase II ascorbate dose for the RT phase would be defined as either the highest dose tested in which  $\leq 1$  of 6 participants experienced a DLT or

a dose that consistently sustained plasma ascorbate levels of  $\geq 20$  mmol/L.

For the ADJ phase, the study employed an intra-patient ascorbate dose escalation design until a DLT occurred or a target plasma ascorbate level was achieved ( $\geq 20$  mmol/L). This design utilizes inpatient dose escalation of 25 g to estimate the MTD or identify the dose required to achieve the target plasma ascorbate level of  $\geq 20$  mmol/L. The ADJ phase began 1 month after completing RT and after MRI determined that there was no associated normal tissue injury. The ADJ-phase starting ascorbate dose was the same dose the subject received during the RT phase. The ascorbate inpatient dose escalation design was key in determining the diminishing returns of higher doses of ascorbate (e.g., 100 g, 125 g). This design was selected because it would enable dose evaluation using subjects as their own control (if there was no toxicity for one cycle, then the next cycle would permit an escalation of dose until plasma ascorbate levels  $\geq 20$  mmol/L were reached). For the initial cohorts, it was known that the ADJ ascorbate dose would be greater than the RT ascorbate dose.

DLTs were defined on the basis of published literature (2, 4) as well as the United States prescribing information for TMZ (17) and included the following events regardless of attribution to ascorbate: infection (grade 4), nausea or vomiting (grade 3) despite maximal supportive care, and decreased neutrophil count (grade 4) or platelet count (grade 4; Table 1). In addition, a serious adverse event (SAE; as defined by FDA) with a reasonable attribution to ascorbate also met DLT criteria. The defined RT phase window for evaluation was RT day 1 through the pre-ADJ cycle 1 MRI, whereas the ADJ-phase evaluation period was from cycle 1 day 1 of TMZ through 30 days after the last ascorbate infusion. DLT criteria were consistent across these phases.

AEs were quantified by National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE; Table 1).

Day 1 of study was defined as RT fraction 1, serving as the first day of the RT phase. Treatment continued until disease progression or completion of six ADJ cycles. A schema for the study is provided (Fig. 1).

### Participants

Adults with biopsy-proven glioblastoma were eligible if the recommended treatment was the standard RT/TMZ regimen described by Stupp and colleagues (2). Descriptions of radiation volumes and doses to organs at risk may be found in Supplementary Tables S1 and S2. *IDH1* testing for the R123H mutation was performed by PCR or next-generation sequencing at the University of Iowa Diagnostic Laboratories. The R123H mutation is the most common *IDH1* mutation (accounts for 90% of the known *IDH1* mutations). MGMT promoter methylation status was determined by PCR analysis at ARUP laboratories, a national reference laboratory, as per University of Iowa Hospitals & Clinics clinical practice. Further detailed criteria are provided on ClinicalTrials.gov (NCT01752491).

### Treatment

Standard therapy was congruent with Stupp and colleagues (2). Briefly, RT was delivered to a total dose of 61.2 Gy in 34 fractions at 1 fraction per business day. Concomitant daily TMZ (75 mg/m<sup>2</sup>) was initiated with radiation and finished with RT completion or a maximum of 49 doses. Adjuvant TMZ was prescribed at 150 mg/m<sup>2</sup> daily for 5 days of a 28-day cycle. A

**Table 1.** Adverse events occurring during RT phase across all dose cohorts ( $n = 13$ ) and ADJ phase ( $n = 12$ )<sup>a</sup>

Grade <sup>b</sup>	Subjects experiencing an adverse event, $n$ (%)								
	Radiation phase				Adjuvant phase				
	1	2	3	4	1	2	3	4	
<b>Hematologic</b>									
Anemia	5 (39)	0	0	0	3 (25)	0	0	0	
White blood cell count decreased	1 (8)	4 (31)	0	0	0	1 (8)	1 (8)	0	
Lymphocyte count decreased	0	3 (23)	5 (39)	2 (15)	1 (8)	4 (33)	5 (42)	0	
Neutrophil count decreased	4 (31)	1 (8)	0	0	1 (8)	0	1 (8)	0	
Platelet count decreased	5 (39)	0	0	0	4 (33)	1 (8)	0	0	
<b>Serum chemistry</b>									
Hyperglycemia	2 (15)	1 (8)	1 (8)	0	2 (17)	3 (25)	0	0	
Hypoglycemia	0	0	0	0	2 (17)	3 (25)	0	0	
Hypernatremia	1 (7.7)	0	1 (8)	0	0	0	0	0	
Hyponatremia	5 (39)	0	0	0	1 (8)	0	0	0	
Hypokalemia	4 (31)	0	1 (8)	0	2 (17)	0	0	0	
ALT increased	1 (8)	2 (15)	1 (8)	0	2 (17)	0	0	0	
AST increased	1 (8)	1 (8)	0	0	1 (8)	0	0	0	
<b>Constitutional</b>									
Fatigue	2 (15)	3 (23)	1 (8)	0	2 (17)	2 (17)	0	0	
Headaches	3 (23)	2 (15)	1 (8)	0	2 (17)	3 (25)	0	0	
Nausea	5 (39)	5 (39)	1 (8)	0	5 (42)	4 (33)	0	0	
Vomiting	5 (39)	0	2 (15)	0	5 (42)	1 (8)	0	0	
Dry mouth	8 (62)	0	0	0	9 (75)	1 (8)	0	0	
Hypertension	0	1 (8)	0	0	0	0	1 (8)	0	
Constipation	4 (31)	2 (15)	0	0	6 (50)	2 (17)	0	0	
Fever	3 (23)	1 (8)	0	0	0	0	0	0	
Chills	7 (54)	0	0	0	6 (50)	0	0	0	
<b>Dose-limiting toxicity</b>									
Infection, grade 4	<b>Description</b>							<b>Attribution</b>	
Nausea, grade 3 (despite maximal support)	Life-threatening or urgent intervention required							Any	
Vomiting, grade 3 (despite maximal support)	Inadequate oral intake, or, tube feeding, TPN, or hospitalization is indicated							Any	
Neutrophil count decreased, grade 4	$\geq 6$ episodes in 24 h, or, tube feeding, TPN, or hospitalization is indicated							Any	
Platelet count decreased grade 4	$< 500$ cells/mm <sup>3</sup>							Any	
Serious adverse event	$< 25,000$ cells/mm <sup>3</sup>							Any	
	Death or a life-threatening event, or, causes or prolongs inpatient hospitalization, or, significant disruption of activities of daily life, or, congenital anomaly or birth defect							Possible, probable, or definite to P-AscH <sup>-</sup>	

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; P-AscH<sup>-</sup>, pharmacologic ascorbate; TPN, total parenteral nutrition.

<sup>a</sup>Events are reported as most severe per subject with one incident per subject per event. All adverse events regardless of attribution are reported.

<sup>b</sup>Graded using the Common Terminology Criteria for Adverse Events version 4.03.

one-time TMZ dose escalation to 200 mg/m<sup>2</sup> was allowed at cycle 2 as per prescribing information. All TMZ dose modifications were per FDA-approved TMZ prescribing information. *Pneumocystis* pneumonia prophylaxis was done and antiemetic medication was administered as per institutional guidelines.

During the RT phase, P-AscH<sup>-</sup> was infused 3 times weekly per the assigned dose cohort. During the ADJ phase, P-AscH<sup>-</sup> was infused twice weekly and escalated weekly until a DLT occurred or the target plasma concentration was achieved ( $\geq 20$  mmol/L). Plasma ascorbate concentrations were evaluated at the end of each ADJ cycle; if the concentration of ascorbate in plasma was lower than the target level, the next cycle's dose was escalated.

### Assessments

For the purposes of AE collection and reporting, subjects were deemed evaluable if they received at least one dose of ascorbate. Thus, all participants have been included in AE reporting (Table 1). For dose evaluation and survival evaluation, subjects had to receive the entire radiation prescription, 90% of the prescribed TMZ during radiation, and 90% of the prescribed ascorbate.

AEs were graded utilizing the CTCAE version 4.03. AEs solicited weekly included rash, nausea, vomiting, fatigue, dry mouth, headache, and diarrhea/constipation. During the RT phase, a complete blood count and differential (CBC w/diff), electrolytes,

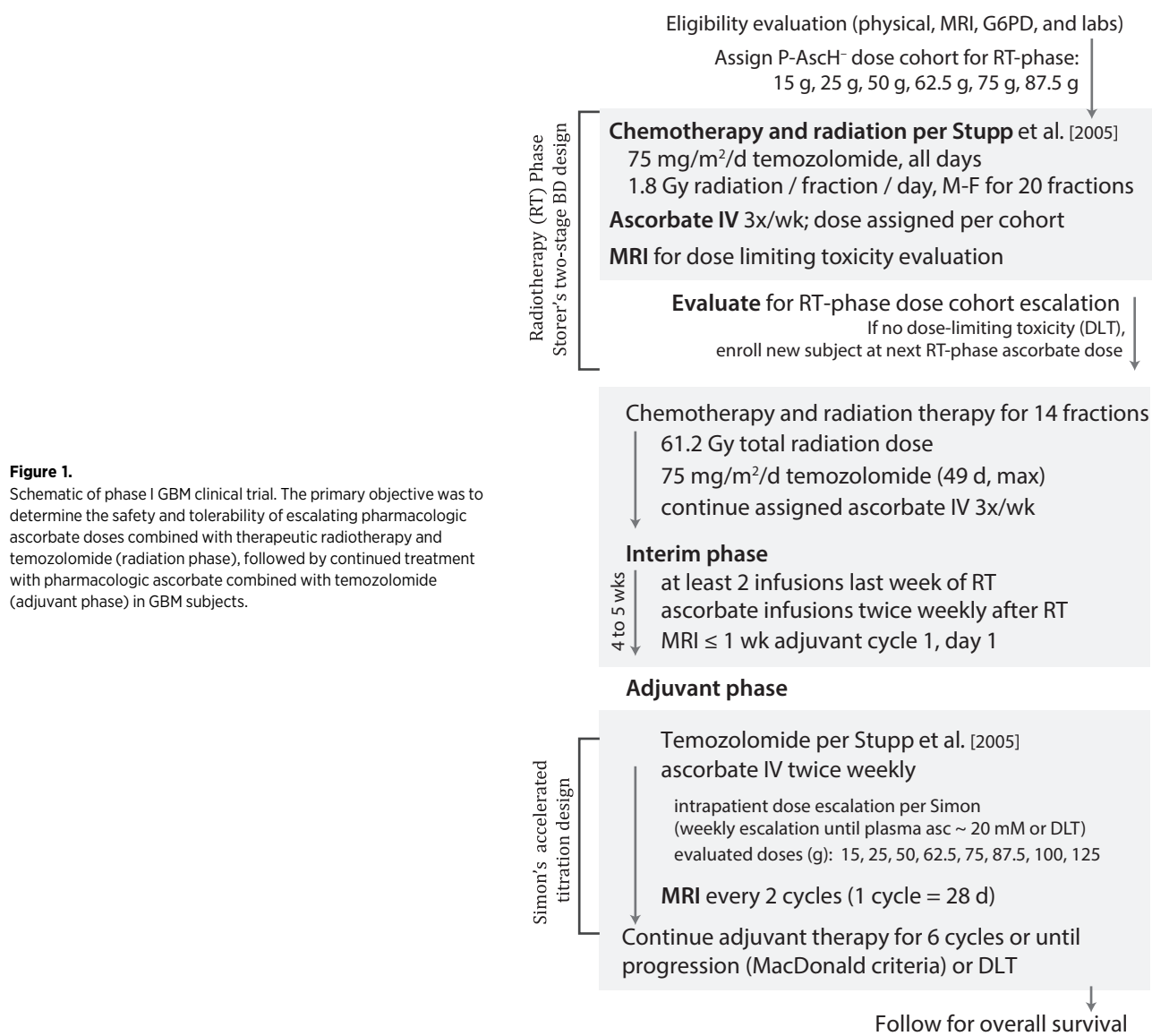
and serum creatinine were obtained weekly with liver function tests obtained every other week during RT phase. During the ADJ phase, a complete metabolic profile and CBC w/diff were obtained at each cycle day 1 with a follow-up CBC w/diff on day 22 for TMZ dose modifications.

Plasma ascorbate levels were assessed weekly during RT, weekly during inpatient dose escalation, and then at the end of each ADJ cycle once the target plasma concentration was achieved. Samples were drawn preinfusion and immediately postinfusion (within 10 minutes). Tumor response was assessed using the Macdonald criteria (18). PFS was calculated as time elapsed from RT fraction 1 to day of progression. OS was calculated as time elapsed from RT fraction 1 to death from any cause or censored at last follow-up.

The systemic oxidative stress marker, 4-hydroxy-2-nonenal (4HNE)-modified proteins, was assessed by dot-blot analysis in plasma samples collected at baseline (before radiotherapy, TMZ therapy, or pharmacologic ascorbate), week 3 of the RT phase, week 5 of the RT phase, and week 7 of the RT phase as described previously (19).

### Statistical analysis

The censor date for these data is October 8, 2018. The primary endpoint was frequency of DLT to estimate MTD as per Storer's

**Figure 1.**

Schematic of phase I GBM clinical trial. The primary objective was to determine the safety and tolerability of escalating pharmacologic ascorbate doses combined with therapeutic radiotherapy and temozolomide (radiation phase), followed by continued treatment with pharmacologic ascorbate combined with temozolomide (adjuvant phase) in GBM subjects.

Design B/D and inpatient dose escalation design (Fig. 2; ref. 16). Eleven subjects were evaluated for DLT analysis. All subjects who received at least one infusion of P-AscH<sup>-</sup> are included in safety analyses. Subjects deemed evaluable by DSMC (i.e., received adequate TMZ, RT, and P-AscH<sup>-</sup>) were included in the efficacy analysis (Fig. 3). Descriptive statistics including medians, ranges, and SDs were calculated.

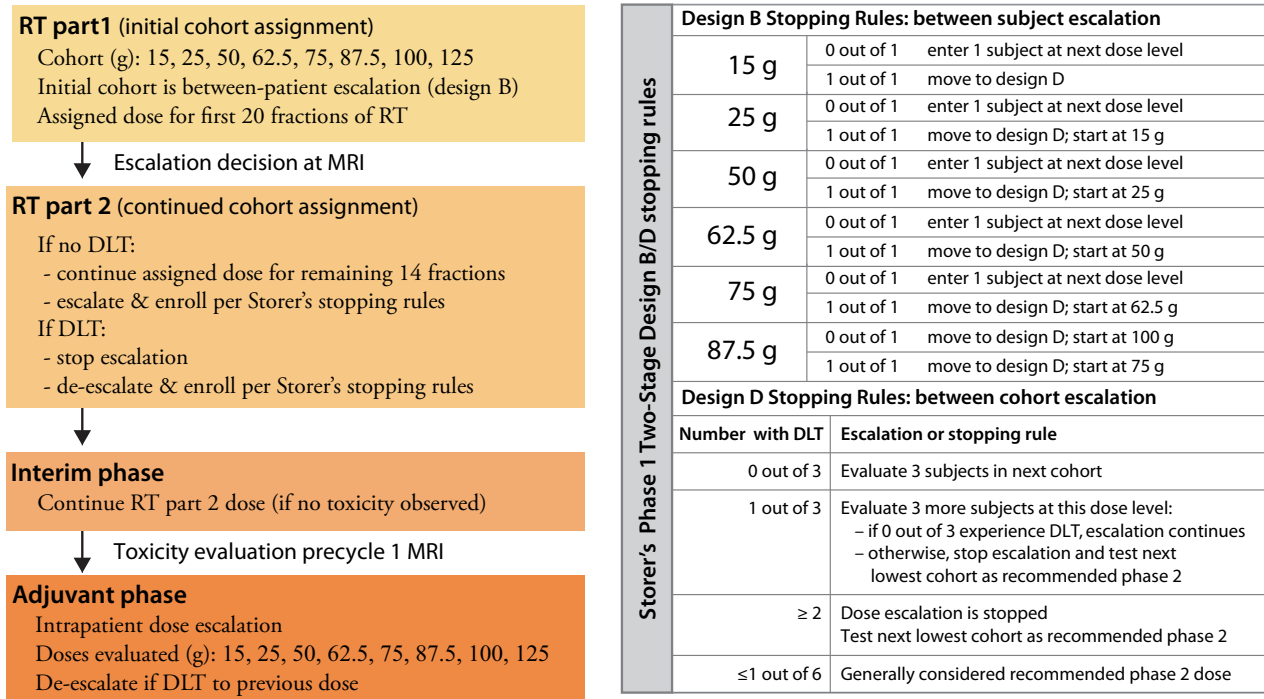
## Results

Thirteen subjects consented to study participation; baseline demographics are summarized in Table 2. Median age at enrollment was 53 years (range of 25–71 years). Seven males and six females were included in the study. Ten patients had maximum safe resection and three had biopsies of tumor. Median time from biopsy or surgery to RT fraction 1 was 3.1 weeks (range of 1.3–4.6 weeks). Of the 11 subjects included in the survival analysis, 8 subjects had no *MGMT* promoter methylation detected. Two subjects had identified *IDH1* mutations.

Two subjects were withdrawn from study. The first 15-g subject developed a pulmonary embolism 3 weeks into the RT phase necessitating a treatment break. The pulmonary embolism was determined to be attributable to a previous medical history of deep venous thrombosis. The DSMC concurred and authorized a second subject at 15 g. The first 50-g subject was withdrawn because of poor tolerance of TMZ due to prior medical history of diverticulitis, which was aggravated by the TMZ. The DSMC determined that this was unrelated to study drug another subject was enrolled to the 50-g cohort. Thus, there are 11 evaluable subjects for primary (safety) and secondary (survival) analysis.

Six P-AscH<sup>-</sup> dose cohorts were evaluated in the RT phase and the interim phase including 15-g, 25-g, 50-g, 62.5-g, 75-g, and 87.5-g ascorbate infusions. DLTs (Table 1) were not realized during the RT phase or interim phase between completing RT and beginning the ADJ phase (1 month; Supplementary Table S3).

Eight P-AscH<sup>-</sup> cohorts were evaluated during the ADJ phase including the RT phase cohorts in addition to 100-g and 125-g ascorbate infusions. During the ADJ phase, all subject ascorbate



**Figure 2.**

Graphical depiction of dose escalation rules applied to the clinical trial. The clinical trial utilized two design methods: Storer's phase I two-stage design B/D (interpatient dose escalation), followed by an inpatient dose escalation to achieve an ascorbate plasma concentration of 20 mmol/L. Dose-limiting toxicities were assessed clinically but also at predefined radiographic checkpoints (fraction 20 of radiation and 1 month postradiation). These checkpoints determined cohort entry as well as stopping rules.

doses were escalated until target plasma ascorbate levels were achieved ( $\geq 20$  mmol/L; ref. 13). The 87.5-g dose consistently achieved plasma ascorbate concentrations of  $\geq 20$  mmol/L in both the RT and ADJ phases. The 100-g and 125-g doses did not significantly increase plasma ascorbate concentrations above 20 mmol/L. The increase in infusion time (+30 minutes per 25 g) was also considered. Thus, 87.5 g was identified as the recommended phase II ascorbate dose during both the RT phase and the ADJ phase.

Of the 11 evaluable subjects, 2 withdrew during the ADJ phase: 1 to enter into another clinical trial and the other to return to family out of state (subjects with red lines in Fig. 3). Three subjects were withdrawn due to disease progression during ADJ therapy phase. Six subjects completed all prescribed ADJ cycles. On average, subjects received 188 days of ascorbate out of a maximum 245 days. The most common reason for withdrawal was disease progression (Fig. 3; Supplementary Table S4).

Overall, there were 85 AEs (CTCAE grades 1, 2, 3, and 4) experienced during the RT phase; due to the exhaustive listing of grade 1 and 2 constitutional events, not all are listed in Table 1. In review, subjects ( $n = 6$ ) assigned to the recommended phase II dose (R2PD) cohort of 87.5 g experienced similar frequency of AEs compared with subjects ( $n = 7$ ) assigned to lower doses. Specifically, the R2PD cohort had 41 grade 1, 21 grade 2, 7 grade 3, and 1 grade 4 AEs. The other lower dose subjects totaled 44, 22, 9, and 2, AEs, respectively.

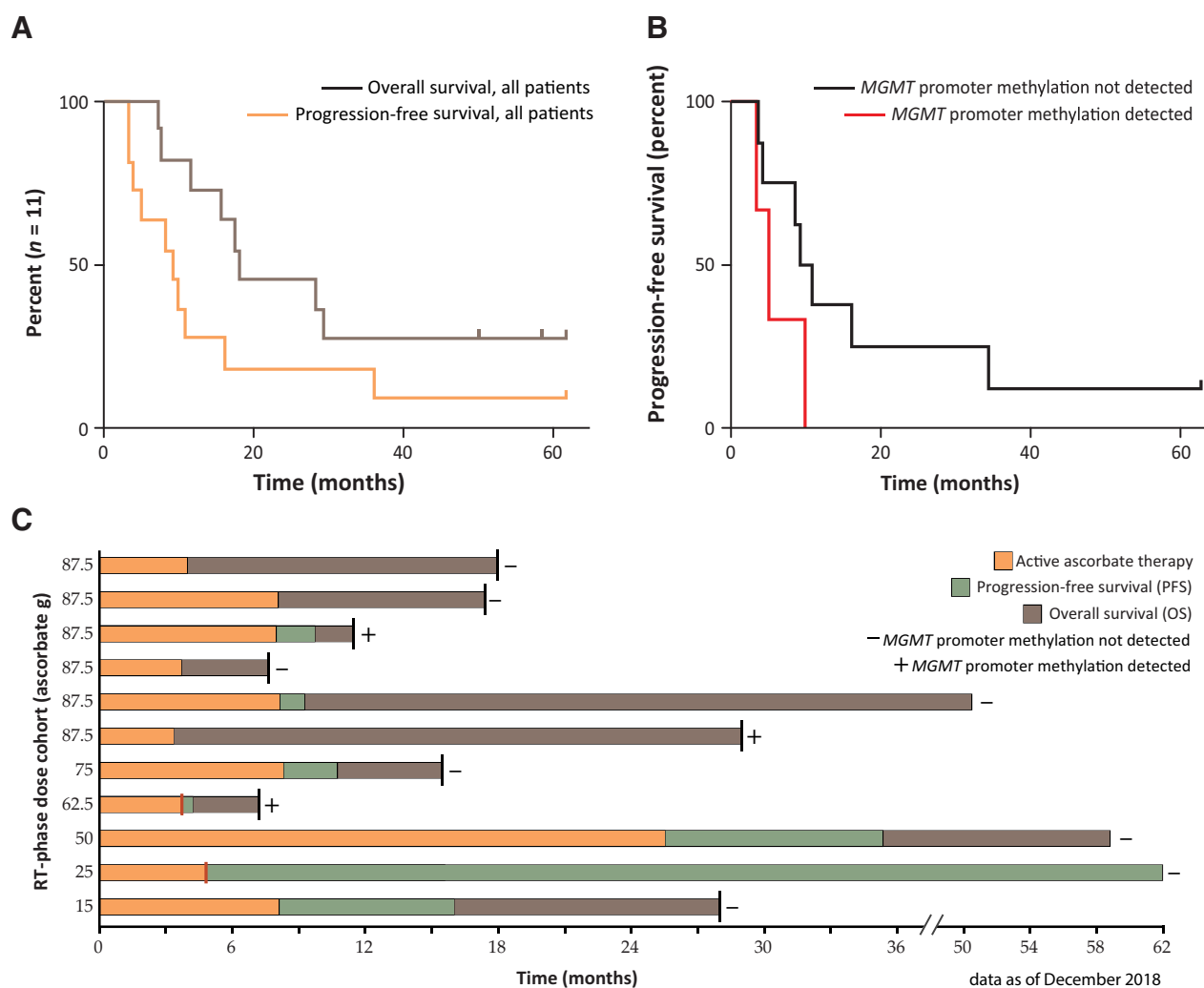
AEs directly attributable to the ascorbate infusions were dry mouth and chills (Table 1). Dry mouth occurred in 75% of the subjects and was thought to be related to the osmotic shift caused

by the salt content of the infusion. Subjects reported that the symptom resolved about an hour postinfusion. Chills were reported in about 54% of the subjects; it is suspected that the temperature (4°C) of the P-AsCH<sup>-</sup> infusion fluid was the cause. Hypokalemia grade 3 (2.9 mEq/L; lower limit of normal = 3.5 mEq/L) not requiring inpatient admission was reported in 1 subject, occurring during the RT phase and was not associated with background therapy or the subject's medical history and was therefore associated to P-AsCH<sup>-</sup>.

The most frequent, severe hematologic AEs occurring during the RT phase were decreased lymphocyte counts (Table 1). In addition to hypokalemia, the most frequent, severe chemistry AEs were single instances of hyperglycemia (grade 3), hypernatremia (grade 3), and elevated aspartate aminotransferase (grade 3). None of these events were believed to be related to P-AsCH<sup>-</sup>. The most frequent, severe constitutional AE was vomiting (Table 1) and this was considered most likely related to standard TMZ chemotherapy.

The observed hematologic events during the ADJ phase were similar with only a single grade 3 absolute neutrophil count decrease. There was also an absence of  $\geq$  grade 3 fatigue, nausea, vomiting, and pain during the ADJ phase (Table 1).

A total of four SAEs occurred during the clinical trial. Of these, three were not attributed to P-AsCH<sup>-</sup> but instead were consistent with prior medical history (pulmonary embolism, diverticulitis) or anticipated with GBM (headache requiring inpatient pain management). The remaining SAE was a seizure that occurred immediately after the screening test dose of 15 g. Upon admission, the subject was found to be febrile (grade 1) and have a



**Figure 3.**

Overall and progression-free survival of phase I GBM clinical trial subjects. Historical median progression-free survival for patients with GBM treated with radiation and temozolomide therapy is approximately 7 months and median overall survival is 14 to 16 months. **A**, Kaplan-Meier progression-free survival curve (orange line) and overall survival curve (gray line) of all subjects treated with pharmacologic ascorbate in combination with therapeutic radiation and temozolomide. **B**, Kaplan-Meier progression-free survival curve of subjects without detection of *MGMT* promoter methylation (black line) and with *MGMT* promoter methylation (red line). Subjects were treated with pharmacologic ascorbate in combination with therapeutic radiation and temozolomide. **C**, Swimmers plot showing outcomes of the 11 evaluable subjects. Active ascorbate therapy is indicated by orange bars, progression-free survival is indicated by green bars, and overall survival is indicated by gray bars. The radiation phase ascorbate dose cohort of each subject is indicated along the y-axis. Red bars indicate subjects who chose to terminate the trial prematurely for personal reasons. Death is indicated by a black line at the end of the bar. Subjects with undetectable *MGMT* promoter methylation are indicated with a minus sign whereas subjects with detectable *MGMT* promoter methylation are indicated with a plus sign. The 62.5-g dose cohort subject and 87.5-g dose cohort subject who remain alive had isocitrate dehydrogenase (IDH) mutations.

pseudomeningocele. Although it was believed that the seizure etiology was most likely fever or the primary GBM postsurgery, P-AscH<sup>-</sup> was attributed as possibly contributing due the contemporaneousness of the event. The subject asked to undergo another 15-g test dose and tolerated the second infusion. The subject completed all therapy without further issues.

Median PFS ( $n = 11$ ) was 9.4 months (95% CI: 5.1–NA; range: 3.4–63 months; the upper 95% confidence limits for the medians are not estimable (NA) because the upper confidence limits of the Kaplan-Meier curves do not drop below 50% survival). Median OS ( $n = 11$ ) was 18 months (95% CI: 16–NA; range: 7.3–63 months). Currently, 3 subjects remain alive. Of these, 1

subject has no evidence of disease progression with last prescribed therapy in March of 2014 (Fig. 3). The remaining 2 experienced disease progression and are receiving salvage therapy (Fig. 3). Historical data demonstrate that patients without *MGMT* promoter methylation have a worse prognostic outcome relative to those with a methylated *MGMT* promoter (4). In our study, subjects without *MGMT* promoter methylation had a median PFS of 10 months (95% CI: 8.5–NA; range: 3.7–63 months) and median OS of 23 months (95% CI: 18–NA; range: 7.8–63). Furthermore, the 3 subjects who remained alive all had undetectable *MGMT* promoter methylation (Fig. 3) and 2 were IDH wild-type. Overall response rate did not vary according to sex or age.

**Table 2.** Baseline characteristics of study patients at enrollment

Characteristic	n = 13 <sup>a</sup> (accrued)	n = 11 <sup>a</sup> (evaluable)
Age, years, median (range)	53 (25–71)	53 (25–68)
Gender, male, n (%)	7 (54)	6 (45)
Race, white, n (%)	13 (100)	11 (100)
Ethnicity, non-Hispanic, n (%)	13 (100)	11 (100)
Karnofsky Performance Status, n (%)		
70	4 (31)	2 (18)
80	3 (23)	3 (27)
90	5 (38)	5 (45)
100	1 (8)	1 (9)
Preoperative lesion location, n (%)		
Frontal	5 (38)	5 (45)
Temporal	5 (38)	3 (27)
Parietal	2 (15)	2 (18)
Occipital	1 (8)	1 (9)
Extent of resection, n (%)		
Gross	8 (62)	6 (55)
Subtotal	2 (15)	2 (18)
Biopsy only	3 (23)	3 (27)
IDH mutation status, n (%)		
Mutant	1 (15)	1 (9)
Wild-type	12 (85)	10 (91)
MGMT promoter methylation, n (%)		
Detected	3 (23)	3 (27)
Not detected	5 (38)	5 (45)
Indeterminant (methylation levels <1%)	3 (23)	3 (27)
Not tested	2 (15)	0 (0)

Abbreviation: IDH, isocitrate dehydrogenase.

<sup>a</sup>Because of rounding, percentages may not total to exactly 100.

The systemic oxidative stress marker, 4HNE-modified proteins, was assessed in select subject plasma samples prior to and during the RT phase. In these subjects, 4HNE-modified proteins decreased over the course of treatment supporting the hypothesis that pharmacologic ascorbate acts as an antioxidant systemically and may protect against radiation and chemotherapy-induced toxicity (Supplementary Fig. S1; ref. 20).

## Discussion

This study reports the first-in-human combination of pharmacologic ascorbate, RT, and TMZ therapy. Data suggest that P-AsCH<sup>-</sup> is safe when combined with RT and TMZ, with minimal toxicity relative to standard RT/TMZ therapy. Although not powered to detect changes in effect size, data suggest that P-AsCH<sup>-</sup> may enhance RT/TMZ effectiveness in patients with GBM, improving both PFS and OS, especially in subjects with undetectable MGMT promoter methylation and IDH wild-type status. Results are sufficiently promising to merit a phase II investigation.

Several additional early-phase clinical trials have found P-AsCH<sup>-</sup> to be safe when combined with chemotherapy and may reduce cancer therapy-associated normal tissue injury (9–11, 13). In stage III or IV ovarian cancer, pharmacologic ascorbate combined with conventional carboplatin and paclitaxel demonstrated reduced incidence of gastrointestinal and hematopoietic toxicity while trending toward improved OS and time to disease progression (10). Similarly, patients with stage IV pancreas cancer treated with pharmacologic ascorbate and gemcitabine demonstrated the safety and tolerability of ascorbate combined with cancer therapy with a suggestion of improved PFS and OS (9). In locally advanced pancreatic cancer, pharmacologic ascorbate combined

with RT and gemcitabine showed an increase in PFS and a trend toward an increase in OS (11).

To our knowledge, this clinical trial was the first to combine P-AsCH<sup>-</sup> with RT and TMZ for a primary brain tumor. Compared with RT/TMZ alone for GBM therapy (2, 4), P-AsCH<sup>-</sup> did not seem to increase hematologic toxicity but instead may provide protective effect. For example, grade 3 or 4 thrombocytopenia was not observed in this small study but had an 11% incidence in TMZ-alone treatment as reported by Stupp and colleagues (2) and 5% reported incidence in the Temodar-prescribing information (3). The other hematologic toxicities reported were all consistent with the effects of TMZ. Data submitted to the FDA identify a common grade  $\geq 3$  reaction as fatigue (13%); yet, in contrast, the single grade 3 fatigue experienced in this study was related to diverticulitis (with the subject having a prior medical history). Protection against neurocognitive decline was not assessed as part of the phase I study but will be considered in subsequent clinical trials. These initial data suggest that P-AsCH<sup>-</sup> may increase RT and TMZ therapeutic tolerability, increasing a subject's quality of life and decreasing therapeutic toxicity (21).

Median PFS ( $n = 11$ ) was 9.4 months (95% CI: 5.1–NA; range: 3.4–63 months; the upper 95% confidence limits for the medians are not estimable (NA) because the upper confidence limits of the Kaplan–Meier curves do not drop below 50% survival.). Median OS ( $n = 11$ ) was 18 months (95% CI: 16–NA; range: 7.3–63 months). Currently, 3 subjects remain alive. Of these, 1 subject has no evidence of disease progression with last prescribed therapy in March of 2014 (Fig. 3). The remaining 2 experienced disease progression and are receiving salvage therapy (Fig. 3). Historical data demonstrate that patients without MGMT promoter methylation have a worse prognostic outcome relative to those with a methylated MGMT promoter (4). In our study, subjects without MGMT promoter methylation had a median PFS of 10 months (95% CI: 8.5–NA; range 3.7–63 months) and median OS of 23 months (95% CI: 18–NA; range 7.8–63). Furthermore, the 3 subjects who remained alive all had undetectable MGMT promoter methylation (Fig. 3) and 2 were IDH wild-type. Overall response rate did not vary according to sex or age.

Median OS ( $n = 11$ ) was 18 months and median PFS was 9.4 months. These survival data compare favorably with historical GBM patients treated with radiation and TMZ therapy alone with a median OS of 14.6 months and PFS of 7 months (2). Furthermore, historical GBM patients with an unmethylated MGMT promoter treated with radiation and TMZ therapy have a median OS of only 12.7 months (22). In the 8 subjects enrolled in this clinical trial with undetectable MGMT promoter methylation ("–" sign in Fig. 3), median OS was 23 months and median PFS was 10 months. Currently, of the 3 subjects who remained alive, 2 had an unfavorable unmethylated MGMT promoter and wild-type IDH1 mutation (Fig. 3).

We found P-AsCH<sup>-</sup> to be safe when combined with RT/TMZ in the treatment of newly diagnosed GBM. The most frequent AEs were transient dry mouth and chills, which were mild and associated acutely with infusion. Based on the encouraging nature of these data, a phase II clinical trial was initiated to assess the efficacy of P-AsCH<sup>-</sup> in GBM subjects treated with RT/TMZ (NCT02344355).

## Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.



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