Title

ABTC-0904: targeting glioma stem cells in GBM: a phase 0/II study of hedgehog pathway inhibitor GDC-0449

Journal

Journal of Neuro-Oncology, 161(1)

ISSN

0167-594X

Authors

Sloan, Andrew E
Nock, Charles J
Ye, Xiaobu
et al.

Publication Date

2023

DOI

10.1007/s11060-022-04193-3

Peer reviewed

HHS Public Access

Author manuscript

J Neurooncol. Author manuscript; available in PMC 2024 June 25.

Published in final edited form as:

J Neurooncol. 2023 January ; 161(1): 33–43. doi:10.1007/s11060-022-04193-3.

ABTC-0904: targeting glioma stem cells in GBM: a phase 0/II study of hedgehog pathway inhibitor GDC-0449

Andrew E. Sloan[1], **Charles J. Nock**[2], **Xiaobu Ye**[3], **Robert Buerki**[4], **Susan Chang**[5], **Glenn Lesser**[6], **Andrew Norden**[7], **Timothy Cloughesy**[8], **Jeffrey Olson**[9], **Amber Kerstetter-Fogle**[1], **Jeremy Rich**[10], **Joy Fisher**[3], **Serena Desideri**[3], **Naoko Takebe**[11], **William Timmer**[11], **Stuart Grossman**[3], **Michael Prados**[5]

[1]Chief of Neuroscience, Piedmont Healthcare, Atlanta, USA

[2]Department of Medicine, UH-Seidman Cancer Center and Case Comprehensive Cancer Center, Cleveland, OH, USA

[3]Adult Brain Tumor Consortium, Department of Medicine, Johns Hopkins School of Medicine, Baltimore, MD, USA

[4]Department of Neurology, UH-Seidman Cancer Center and Case Comprehensive Cancer Center, Cleveland, OH, USA

[5]Department of Neurosurgery, University of California, San Francisco, San Francisco, CA, USA

[6]Department of Radiation Oncology, Wake Forest University, Wake Forest, NC, USA

[7]Department of Medicine, Dana Farber Cancer Institute, Boston, MA, USA

✉Andrew E. Sloan, Andrew.Sloan@Piedmont.org.

Author contributions Study conception and design: SG, AES, MP, NT, WT. Clinical Protocol: AES, CJN, and SG. Development of Biomarker Studies: AES. Surgical Training of investigators: AES. Statistical design of study: Lamborn, XY. Conduct of clinical studies: CJN, AES, SG, MP. Performance of PK and PD studies: Graham, Supko. Performance of neurosphere assays, PCR, and microscopy: AES, AK-F. Analysis and interpretation of data: AES, CJN, Barnholtz-Sloan, AK-F, XY, Rich, SG, Prados. Drafting the article: AES, MP, Nock, Rich, SG. Critically revising the article: all authors. Patient care: AES, CJN, SG, MP.

Declarations

Conflict of interest The authors have no conflict of interests.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11060-022-04193-3.

Ethical approval Authors of research involving human or animal subjects should include a statement that confirms that the study was approved (or granted exemption) by the appropriate institutional and/or national research ethics committee (including the name of the ethics committee and reference number, if available)…

Consent to participate For all research involving human subjects, freely-given, informed consent to participate in the study must be obtained from participants (or their parent or legal guardian in the case of children under 16) and a statement to this effect should appear in the manuscript.

Informed consent Informed consent was obtained from all individual participants included in the study. Written informed consent was obtained from the parents.

Consent to publish Individuals may consent to participate in a study, but object to having their data published in a journal article. If your manuscript contains any individual person's data in any form (including any individual details, images or videos), consent for publication must be obtained from that person, or in the case of children, their parent or legal guardian. This is in particular applicable to case studies. A statement confirming that consent to publish has been received from all participants should appear in the manuscript.

Research involving human and animal rights The authors affirm that human research participants provided informed consent for publication of the images in Fig. 1a–c.

[8]Department of Neurology, University of California, Los Angeles, Los Angeles, CA, USA

[9]Department of Neurosurgery, Emory University, Atlanta, GA, USA

[10]Department of Neurology and Hillman Cancer Center, University of Pittsburgh, Pittsburgh, PA, USA

[11]National Cancer Institute, Clinical Investigations Branch, National Institutes of Health, Bethesda, MD, USA

Abstract

Purpose—Gliomagenesis and resistance of glioblastoma (GBM) are believed to be mediated by glioma stem cells (GSC). Evidence suggests that SHH signaling promotes GSC proliferation and self-renewal.

Methods—ABTC-0904 was a two-arm, multicenter phase 0/II study of GDC-0449, an oral inhibitor of Smoothened (SMO) in patients undergoing resection for recurrent GBM. All patients (Arms I and II) had surgery and received drug post-operatively. Only patients in Arm I received drug prior to surgery. The *primary objective* was to determine 6-month progression free survival (PFS-6). Secondary endpoints include median PFS (mPFS) and overall survival (mOS), response rate, and toxicity. *Correlative studies* included bioanalysis of GDC-0449, and inhibition of SHH signaling, GSC proliferation and self-renewal.

Results—Forty-one patients were enrolled. Pharmacokinetics of GDC-0449 in plasma demonstrated levels within expected therapeutic range in 75% of patients. The proportion of tumorcells producing $CD133^+$ neurospheres, neurosphere proliferation, self-renewal, and expression of the SHh downstream signaling was significantly decreased in Arm I following GDC-0449 treatment ($p < 0.005$; $p < 0.001$ respectively) compared to Arm II (no drug pre-op). Treatment was well tolerated. There were no objective responders in either arm. Overall PFS-6 was 2.4% (95% CI 0.9–11.1%). Median PFS was 2.3 months (95% CI 1.9–2.6) and mOS was 7.8 months (95% CI 5.4–10.1).

Conclusions—GDC-0449 was well tolerated, reached tumor, and inhibited $CD133^+$ neurosphere formation, but had little clinical efficacy as a single agent in rGBM. This suggests growth and maintenance of rGBM is not solely dependent on the SHH pathway thus targeting SMO may require combined approaches.

Keywords

Glioblastoma; Glioma stem cells (GSC); Hedgehog (SHH) signaling pathway; GDC-0449; Phase 0/II Clinical Trial

Introduction

Glioblastoma (GBM) is the most common malignant CNS cancer in adults with a median survival less than one year. Recent studies suggest that both GBM oncogenesis and development of resistance to chemo-radiotherapy are driven by glioma stem cells (GSC) [1–5].

Embryonic developmental signaling pathways, such as sonic hedgehog (SHH), are implicated in treatment resistance, maintenance of stemness, and proliferation in GSCs [5–8]. The oral drug GDC-0449 inhibits the Smoothened (SMO) receptor and thus SHH mediated signaling in GSCs via Gli1-Gli3 transcription factors. GDC-0449 is FDA-approved for treatment of advanced basal cell carcinoma but activity in GBM is unknown [9, 10]. We hypothesized that suppressing the SHH pathway with GDC-0449 in the glioma stem cell population of rGBM would slow tumor progression and improve survival. To assess the biological as well as the clinical efficacy of GDC-0449, we performed a randomized, open label phase 0/II trial of GDC-0449 in rGBM patients undergoing surgical debulking of recurrent tumor. Patients in Arm I (pre-operative treatment) received GDC-0449 for 7 days prior to surgery; patients in Arm II were untreated. Patients on both arms underwent surgery; all patients received GDC-0449 post-operatively until progression. Resected tumor tissues were used for correlative studies, including pharmacokinetic (PK) and pharmacodynamic (PD) biomarker analyses, quantitative assessment of $CD133^+$ neurosphere generation, proliferation, and self-renewal properties of these cells, and quantitation of GLI signaling activity.

Materials and methods

Study conduct

ABTC-0904 was a phase 0/II, open-label, study for surgically-resectable adult rGBM at eight ABTC member institutions between 2010 and 2011. All study procedures were IRB-approved. Adult patients with a previous histologically-confirmed diagnosis of GBM were eligible. Patients must have failed prior radiation with or without prior chemotherapy, with measurable contrast enhancing disease at recurrence and be candidates for repeat resection with additional entry criterion listed (clinicaltrials.gov (NCT00980343). The intent of surgery was maximal safe resection with a minimum of 2.5 cm of tumor available for biomarker analyses (PK and PD). Archived formalin-fixed paraffin-embedded tissue from the initial resection at the time of diagnosis, prior to any treatment was also required. Patients were sequentially assigned *to either* receive GDC-0449 (Arm I) pre-operatively for 7 days or to receive no drug (Arm II) prior to surgical resection (Fig. 1). All patients were to receive GDC-0449 within 28 days post-operatively and then to continue until intolerance, progression, death, or withdrawal of consent. Treatment consisted of 150 mg GDC-0449 continuous daily dosing by mouth (dosing based on a prior phase I study [11] and equivalent to the FDA-approved dosing for basal cell carcinoma) in 28-day cycles. Assessment of response included clinical and neurological exams as described in detail on both Adult Brain Tumor Consortium (ABTC) and ClinicalTrials.gov websites (NCT00980343). Toxicity and treatment-emergent adverse events were determined by CTCAE-4.0 (Common Terminology Criteria for Adverse Events; https://ctep.cancer.gov/). OS and PFS were calculated according to the statistical approach outlined below using Kaplan–Meier survival analysis software R, v2.10.0.

Statistics

The primary objective of the trial was to assess 6-month progression free survival (PFS-6) measured from start of patient registration till progression or death. The secondary

clinical objectives were toxicity, radiographic response rate, median progression-free survival (mPFS), and median overall survival (mOS). Correlative endpoints included pharmacokinetics and pharmacodynamics of GDC-0449 in both serum and resected tumor in Arm I; assessment of the formation, proliferation, and self-renewal of C $D133^+$ neurospheres and assessment of the components of the SHH signaling pathway by quantitative RT-PCR (q-RT-PCR) in both Arm I and Arm II. An exploratory endpoint was to correlate clinical outcome (PFS-6) with biologic markers. With 40 patients, there was 90% power to detect an improvement in PFS6 from 10% (the rate historically seen for agents felt to be ineffective) to 25%. This assumes use of alpha = 0.1 one-tailed. For the purpose of the primary assessment, a patient was considered a success only if the patient was documented to have been progression-free at 6 months without additional therapeutic interventions. Median PFS and survival times were estimated using Kaplan–Meier curves. Response rates and toxicity incidences were estimated based on the binomial distribution. Given the potential for ineligible cases, we planned to screen 45 patients in order to achieve 40 evaluable cases.

In addition to the clinical assessments, the study was intended to address exploratory laboratory correlates related to potential biomarkers of glioma stem cells (GSCs). Two laboratory hypotheses were identified in advance as primary and the study sample size was determined to assure adequate information to address correlative questions. Specifically, these included: (a) The frequency, proliferation, and self-renewal capacity of tumor-derived *CD133*+ neurospheres in treated patients (Arm I) will be less than that observed in patients not treated with (Arm II); (b) SHH pathway genes and markers of proliferation will be decreased in treated (Arm I) relative to untreated (Arm II) patients. With 20 patients in each of the 2 surgery groups and doing a direct comparison with Fisher's exact test (alpha = 0.05 1-sided) there would be 90% power to detect a reduction in development of neurospheres from 70 to 20% and a reduction in activated pathway markers from 80 to 30%. Analysis of laboratory data used estimation/hypothesis testing based on the binomial distribution for categorical data.

Laboratory methods

Tumor specimens were acquired, processed, and transported according to specified standard operating procedures (SOPs) as specified in the trial protocol and addendum (NCT00980343). Summaries of these procedures are described below. More detailed procedures are provided in the Supplemental Methods and Figures. All specimens were transferred to the Translation Research Core (TRC) at the Case Comprehensive Cancer Center (CCCC) either fresh or by overnight courier in a specially designed refrigerated package.

GDC-0449 bioanalysis in plasma and in tumor (Arm I)

Surgery, during which tumor tissue samples was acquired, was performed at least 24 h after taking the last dose of GDC-0449 (Arm I); thus GDC-0449 was not taken on the day of surgery. Plasma concentrations of GDC-0449 were determined from two single specimens of whole blood collected in K2-EDTA tubes just prior to and immediately after the surgical procedure, and collection times recorded. Intra-tumor concentrations of GDC-0449 were obtained from a single 100–150 mg specimen of fresh tumor within the enhancing portion

of the tumor, avoiding any obvious necrotic regions. The two frozen serum samples and the tissue samples were then transferred *in batch* to Tandem Laboratories where plasma and tissue levels of GDC-0449 were quantified by HPLC with electrospray ionization mass spectrometry as previously described [11].

Neurosphere studies (arm I and arm II)

Neurosphere (NS) preparation and analysis, proliferation assay, and limited dilution assay were performed as described in previous publications [12–20]. Tissue and NS preparation and analysis was performed by technicians and scientists blinded to the trial design, patient characteristics, and treatment conditions.

Proliferation assays

$CD133^+$ NS were plated at a density of 1000 cells/well in a 96-well plate in triplicate as previously described [18]. Cell number was measured every other day and normalized to the initial reading for 5 consecutive days using the CellTiter-Glo assay kit [21].

In vitro limiting dilution neurosphere formation assay

Various numbers of glioblastoma cells were seeded in 96-well plates containing 100 μl completed neurobasal medium. After 14 days, the neurospheres were measured, and were analyzed by Extreme Limiting Dilution analysis software (http://bioinf.wehi.edu.au/software/elda).

Immunocytochemistry and fluorescence microscopy

$CD133^+$ neurospheres were washed with 1% PBS and resuspended in supplemented Dulbecco's Modified Eagle's Medium (DMEM-Low Glucose), fixed with 4% paraformaldehyde. Prior to coverslip application, nuclei were counterstained with DAPI and imaging done using a Leica SP-5 confocal microscope as described previously [21, 22].

Molecular analysis of neurospheres

Further molecular analysis of the neurospheres was also performed when adequate material was present. This included: (a) Analysis of MGMT promotor methylation, which was performed by the Genomics Core of the Case Comprehensive Cancer Center using a methylation-specific polymerase chain reaction—based assay as described [23]; (b). RNA sequencing, which was performed by the Genomics core of the Case Comprehensive Cancer Center for sequencing using the Illumina TruSeq Stranded Total RNA Library Prep Kit; as well as (c) in silico analyses, which was used to classify neurospheres into proneural, classical, and mesenchymal subtypes as previously described in the literature [24, 25].

Array comparative genomic hybridization (cGH)

Genomic DNA (~ 0.5 μg) from GSCs isolated from CD 133^+ NS from three patients as well as control neural stem cells were fluorescently labeled with Cy3 and Cy5 labeled random nanomers. DNA was then fragmented at 98 °C for 10 min, and amplified with Klenow fragment (3′–5′ exo-). The Cy5-labeled DNA (5 μg) was co-hybridized with the Cy3-labeled human male reference DNA (5 μg) on Agilent high density microarray

using the hybridization and washing conditions from the Agilent Oligonucleotide Array-Based CGH protocol (v6.2) for Genomic DNA Analysis. Arrays were scanned with the Agilent DNA Microarray Scanner at a 3um scan resolution, and quantified with Feature Extraction 11.0.1.1. BioDiscovery's FASST2 Segmentation Algorithm, a Hidden Markov Model (HMM) based approach, was used to make copy number calls. All samples are corrected for GC wave content using systematic correction algorithms.

Results

Clinical findings

Patient characteristics—A total of 45 patients were screened and underwent surgery for presumed rGBM. Of these, 4 patients (1 in Arm I and 3 in Arm II) were deemed unevaluable as the majority of the resected tissue removed was deemed consistent with radiation treatment effect (radiation necrosis) and did not receive further study drug, resulting in 41 evaluable patients (21 in Arm I, 20 in Arm II) who are the subject of this analysis. These 4 cases were also not evaluated for clinical outcome or biomarker analyses. Patient demographic characteristics are described in Table 1. Each study arm was equally matched across demographic criteria.

Safety—All 41 evaluable patients were evaluated for safety. GDC-0449 was well-tolerated without any grade ≥ 4 serious adverse events attributable to study drug. Table 2A and B illustrate grade 1–3 adverse events and its attribution to GDC-0449 in Arms I and II, respectively. There was no significant difference between toxicity in the two study Arms.

Imaging response—At the time of protocol development and activation, treatment responses were determined using the MacDonald Criteria [26]. Given the low overall response rate and progression-free survival, repeat review using the RANO criteria was not performed. Patients with residual measurable disease following surgery were assessed for objective response. No complete radiologic (CR) or partial responses (PR) were observed. There were 8 patients with stable disease (SD), and 23 had progressive disease (PD). There were 10 patients who were not evaluable for objective treatment response assessment by imaging due to lack of residual measurable disease post-operatively.

Effect of GDC-0449 on rGBM mOS and PFS—The primary endpoint, PFS-6, measured from the day of registration in both arms pre-operatively, was 2.4% (95% CI 0.9–11.1%). The PFS-6 rate (21 patients) was 0% (95% CI 0–13.3%) in arm I, and 5% (95% CI 1.8–21.6%) in Arm II (20 patients). Overall median PFS was 2.27 months (95% CI 1.9–2.6) and median OS was 7.8 months (95% CI 5.4–10.1 mos.; Fig. 2A and B respectively). There was no significant difference in PFS or OS between the two arms ($p = 0.98$; $p = 0.37$).

Biomarker studies

Pharmacokinetics and pharmacodynamics of GDC-0449—Recurrent GBM tumor specimens (mean mass 4.5 g; range 0.9–30.1 g) from 8 centers in the United States were obtained from 41 patients. Mean time from OR to processing was 20.5 h (range 5.0–21 h). One patient in Arm I had predominantly treatment effect with only a small amount of

viable tumor and was not evaluated for tumor PK or PD studies. Patient 3 in Arm II was not evaluable for PD studies due to unanticipated delay in receipt of the tumor sample leaving no viable tissue by the time it reached the lab. Thus, the PK and PD studies were done in 20/21 cases in Arm I and the PD studies in 19/20 cases in Arm II. Neurosphere, proliferation and limited dilution assay was also not performed in patient 3 in Arm II due to lack of viable tissue. Mean viability by trypan-blue exclusion was 71.3% (range 61.1–92.2).

Plasma and intra-tumoral concentrations of GDC-0449 from the 20 patients in Arm I with rGBM are shown in Supplementary Table 1. The pharmacokinetics of GDC-0449 in patients with refractory solid tumors, including basal cell carcinoma, pancreatic cancer, and medulloblastoma have been performed previously [11, 27, 28]. Plasma concentrations of GDC-0449 either at the start (T1) or end of surgery (T2) were at or above 7300 ng/ml (17.3 micromoles/l) in 15/20 (75%) of patients in Arm I of our trial Using the mean plasma concentration from both time points resulted in 13/20 (65%) achieving this threshold. This corresponds to the median day 7 plasma concentration determined to be effective in patients in a previously published phase I trial of GDC-0449 [9]. GDC-0449 plasma levels in 9 of 20 patients in Arm I (45%) were at or near 9269 ng/ml (22 μM), plateau levels achieved on day 21 of a previous pharmacokinetic trial [11], and which is comparable to GDC-0449 steady state levels from previous studies of 22.6 ± 10.8 μM [9]. Intratumoral levels reached tissue concentrations that significantly exceeded the free I C_{95} dose (17.7 ng/ml or 0.042 uM of GDC-0449) threshold that achieved inhibition of Gli-1 mRNA in a medulloblastoma xenograft model [27] in 9/20 (45% %) evaluable patients (Supplementary Table 1).

Effect of GDC-0449 on CD133$^+$ neurosphere formation—Primary cell cultures of GBM tumors derived from patients in Arm I and Arm II of the study were dissociated in vitro in serum free media to determine the numbers of cells that had generated neurospheres (Supplementary Fig. 1). There was inadequate tumor tissue for evaluation from one patient in each Arm. Following pre-treatment with GDC-0449, only 3/20 evaluable tumors (15%) from patients in Arm I yielded CD 133$^+$ NS, while 11/19 evaluable tumors (58%) from untreated patients in Arm II yielded CD 133$^+$ NS ($p < 0.005$, Fisher's exact test). The results are is summarized in Supplementary Table 2, and representative photomicrographs of the NS are provided in Supplementary Fig. 1. There was no correlation with neurosphere formation and clinical outcome (data not shown).

Characterization of glioma stem cells and classification of GBM tumors—Molecular genotype profiles of GSCs from resected tumor were verified using array cGH as described in the Methods. Ex vivo CD133$^+$, Nestin$^+$ GSCs from both Arms I and II had canonical chromosomal aberrations associated with GBM such as loss of heterozygosity (LOH) on chromosome 10 and gain of chromosome 7. In contrast, control neural stem cells (NSC) did not demonstrate gross chromosomal abnormalities characteristic of GBM (Fig. 3).

The rates of proliferation and self-renewal of CD133+ NS cells derived from patients in Arm I (pre-operative treatment with GDC-0449) were not significantly different than that of CD133$^+$ NS cells from patients in Arm II, nor was the expression of nestin, CD-133, or CD-15 (Supplemental Figs. 2 and 3 respectively). The Notably, tumors

forming neurospheres had markedly faster in vitro proliferation and self-renewal in limited dilution assays than tumors not forming neurospheres (Supplemental Figs. 2 and 3 respectively). Neurosphere formation, status of MGMT promoter methylation, and molecular subclassification of the neurosphere are summarized in Supplementary Table 2.

Pharmacodynamic effect of GDC-0449 on SHh pathway—The pharmacodynamic effects of GDC-0449 assessed by q-RT-PCR evaluation of levels of *Gli-1*, *Gli-2*, *Gli-3* and *Ptch-1b* mRNAs in triplicate from NS in the two arms were also compared (Supplementary Fig. 4). RNA isolated from $CD133^+$ NS cells from patients in Arm II (untreated) exhibited increased expression of *Gli-1*, Gli-2 *Gli-3 and Ptch1b*, of 5.0-, 7.1– 10.2- and 5.0-fold high compared to Arm I ($p < 0.001$ to 0.01). Additional analysis failed to reveal any correlation between expression of GLI-1, GLI-2 or GLI-3 and patient survival (data not shown).

Discussion

This multi-center, open-label phase 0/II study failed to achieve the primary endpoint, with no improvement in PFS-6, and a rate of 2.4% which was well below comparable landmarks in the rGBM population, demonstrating that GDC-0449 administered at the standard dose of 150 mg/day did not have clinical benefit in recurrent GBM. The drug was safe, well-tolerated, and achieved what was felt to be therapeutically-adequate plasma concentrations in this population, comparable to that observed in successful phase II trials of non-CNS solid tumors [11]. In 9/20 cases on Arm I, the intra-tumoral concentration of GDC-0449 following pre-operative treatment in rGBM exceeded free IC_{95} levels previously reported to be effective in a subcutaneous murine medulloblastoma xenograft model [27].

GDC-0449 was shown to inhibit the formation and/or maintenance of ex vivo $CD133^+$ neurospheres, a model for tumor-derived GSCs. Patients treated for 7 days pre-operatively had decreased initiation of C $D133^+$ neurospheres without significantly impairing proliferation ability or self-renewal. The size, appearance and surface expression of the neurospheres that did form were indistinguishable from control neurospheres. Molecular profiling of these patient-derived $CD133^+$ NS most commonly demonstrated mesenchymal GBM subtypes, and were characterized by isochromosome 10 and trisomy 7 (consistant with derivation from tumor), rather than non-neoplastic neural stem cells, which did not exhibit these chromosomal aberrations. Expression of SHH pathway transcription factors and feedback loop members Gli-1, Gli-2, and Gli-3 and Ptch1b was markedly diminished in C $D133^+$ NS from patients treated with GDC-0449 pre-operatively (Arm I) compared to those derived from untreated patients (Arm II), indicating effective targeting of SHH signaling in vivo in actual GBM patients.

Despite adequate blood–brain barrier penetration into rGBM tumor cells in nearly 50% of cases, and evidence of SHH downregulation specifically in tumor-derived GSCs, the SHH inhibitor GDC-0449 did not demonstrate evidence of clinical efficacy as a single agent. Treatment with the drug was found to be safe, with minimal treatment-emergent toxicity in the rGBM population. The median PFS of 2.3 months, and median OS of 7.8 months is typical of results from other phase II studies of drugs deemed “inactive” in similarly highly selected patient populations in the ABTC/NABTT/NABTC consortiums,

but is also comparable to predicted mOS in this population with second and third line treatments. This result suggests that targeting only the sonic hedgehog pathway in rGBM, even with agents which are brain-penetrant and pharmacodynamically active, is insufficient to generate meaningful clinical responses. Indeed, recent studies in genetically engineered glioma models suggest that multiple cellular subpopulations, including both stem and non-stem cells, need to be targeted in GBM to achieve a clinically efficacious response [19, 29, 30].

Although this trial did not achieve improvement in PFS-6 or landmark survival outcomes from GDC-0449 targeting the GSC population within rGBM tumors, additional studies may identify biomarkers of response to SHH pathway downregulation and other additional signaling pathways that may promote tumorigenesis and treatment resistance in GBM. Better SMO targeting agents may also be more effective. In particular, senidegib a newer orally bioavailable SMO inhibitor, structurally distinct from GDC-0449, demonstrated a higher response rate and similar side-effect profile compared to GDC-0449 in basal cell carcinoma. GDC-0449 may also be more effective in combination with other agents.

This trial did demonstrate the feasibility of having a multi-disciplinary consortium (ABTC) collect and distribute biological specimens, from which biomarkers and ex vivo GSC can be generated. This study also illustrates the importance of surgical “window of opportunity” phase 0 studies in establishing drug delivery not only across the blood–brain barrier, but also penetrating into actual brain tumors and demonstrating on-target biological activity. Although single-agent treatment of rGBM with GDC-0449 did not reveal clinical benefit, we were able to measure intratumoral PK and PD successfully as well as downstream signaling, providing a model for future investigations of other agents or drug combinations in this malignant tumor. The presence of intratumoral biologic activity of GDC-0449 in rGBM-derived GSCs may suggests synergistic combination of this drug with agents targeting other signaling pathways driving GSC stemness and proliferation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The authors acknowledge the contributions of the patients who participated in this study and their families. We thank Lisa Rogers, DO, formerly from the Seidman Cancer Center, John Pink, Ph.D., and Erin Hohler of the Translational Research Core of the Case Comprehensive Cancer Center (CCCC) for coordinating the biomarker studies, as well as Megan Sims, of ABTC for clinical coordination.

Funding

National Institutes of Health [CA 137443-ARRA]; The Adult Brain Tumor Consortium (ABTC; PI, S.G.; Co-I, A.E.S); The Peter D. Cristal Chair; The Kimble Foundation; The Ferry Family Foundation; The Kaufman Fund (to A.E.S.).

Data availability statement

All data refered to in this manuscript is available for review.

References

1. Singh SK, Clarke ID, Terasaki M et al. (2003) Identification of a cancer stem cell in human brain tumors. Cancer Res 63(18):5821–5828 [PubMed: 14522905]
2. Singh SK, Hawkins C, Clarke ID et al. (2004) Identification of human brain tumour initiating cells. Nature 432(7015):396–401. 10.1038/nature03128 [PubMed: 15549107]
3. Yuan X, Curtin J, Xiong Y et al. (2004) Isolation of cancer stem cells from adult glioblastoma multiforme. Oncogene 23(58):9392–9400. 10.1038/sj.onc.208311 [PubMed: 15558011]
4. Dirks PB (2008) Brain tumor stem cells: bringing order to the chaos of brain cancer. J Clin Oncol 26(17):2916–2924. 10.1200/JCO.2008.17.6792 [PubMed: 18539973]
5. Liu G, Yuan X, Zeng Z et al. (2006) Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. Mol Cancer 2(5):67. 10.1186/1476-4598-5-67
6. Beachy PA, Karhadkar SS, Berman DM (2004) Tissue repair and stem cell renewal in carcinogenesis. Nature 432(7015):324–331. 10.1038/nature03100 [PubMed: 15549094]
7. Bar EE, Chaudhry A, Lin A et al. (2007) Cyclopamine-mediated hedgehog pathway inhibition depletes stem-like cancer cells in glioblastoma. Stem Cells 25(10):2524–2533. 10.1634/stemcells.2007-0166 [PubMed: 17628016]
8. Clement V, Sanchez P, de Tribolet N, Radovanovic I, RuiziAltaba A (2007) HEDGEHOG-GLI1 signaling regulates human glioma growth, cancer stem cell self-renewal, and tumorigenicity. Curr Biol 17(2):165–172. 10.1016/j.cub.2006.11.033 [PubMed: 17196391]
9. Von Hoff DD, LoRusso PM, Rudin CM et al. (2009) Inhibition of the hedgehog pathway in advanced basal-cell carcinoma. N Engl J Med 361(12):1164–1172. 10.1056/NEJMoa0905360 [PubMed: 19726763]
10. Rudin CM, Hann CL, Laterra J et al. (2009) Treatment of medulloblastoma with hedgehog pathway inhibitor GDC-0449. N Engl J Med 361(12):1173–1178. 10.1056/NEJMoa0902903 [PubMed: 19726761]
11. Graham RA, Lum BL, Cheeti S et al. (2011) Pharmacokinetics of hedgehog pathway inhibitor vismodegib (GDC-0449) in patients with locally advanced or metastatic solid tumors: the role of alpha-1-acid glycoprotein binding. Clin Cancer Res 17(8):2512–2520. 10.1158/1078-0432.CCR-10-2736 [PubMed: 21300760]
12. Hjelmeland AB, WuQ HJ et al. (2011) Acidic stress promotes a glioma stem cell phenotype. Cell Death Differ 18(5):529–540
13. Guryanova OA, Wu Q, Cheng L et al. (2011) Nonreceptor tyrosine kinase BMX maintains self-renewal, and tumorigenic potential of gliomblastoma stem cells by activating STAT3. Cancer Cell 19(4):498–511 [PubMed: 21481791]
14. Eyler CE, Wu W, Yan K et al. (2011) Glioma stem cell proliferation and tumor growth are promoted by nitric oxide synthase-2. Cell 146(1):53–66 [PubMed: 21729780]
15. Heddleston JM, Wu Q, Rivera M et al. (2012) Hypoxia-induced mixed-lineage leukemia 1 regulates glioma stem cell tumorigenic potential. Cell Death Differ 19:428–439. 10.1038/cdd.2011.109 [PubMed: 21836617]
16. Kaur H, Phillips-Mason PJ, Burden-Gulley SM et al. (2012) Cadherin-11, a marker of the mesenchymal phenotype, regulates glioblastoma cell migration and survival in vivo. Mol Cancer Res 10(3):293–304 [PubMed: 22267545]
17. Huang P, Sandhya R, Ahluwalia MS et al. (2012) Tumor necrosis factor receptor I expression on tumor-associated endothelial cells in glioblastomas provides a pro-apoptotic signal that is negatively regulated by integrin a681. Cancer Res 72(6):1428–1437 [PubMed: 22396498]
18. Lathia J, Li M, Hall PE et al. (2012) Laminin alpha 2 enables glioblastoma stem cell growth. Ann Neurol 72(5):766–778. 10.1002/ana.23674 [PubMed: 23280793]
19. Jin X, Kim JLY, Wu A et al. (2017) Targeting glioma stem cells through combined BMI1 and EZH2 inhibition. Nat Med 23:1352–1361. 10.1038/nm.4415 [PubMed: 29035367]
20. Li Z, Bao S, Wu Q et al. (2009) Hypoxia-inducible factors regulate tumorigenic capacity of glioma stem cells. Cancer Cell 15(6):501–513 [PubMed: 19477429]

21. Wang J, Wang H, Li Z et al. (2008) c-Myc is required for maintenance of glioma cancer stem cells. PLoS ONE 3(11):e3769 [PubMed: 19020659]
22. Lathia JD, Gallagher J, Heddleston JM et al. (2010) Integrin alpha 6 regulates glioblastoma stem cells. Cell Stem Cell 6(5):421–432 [PubMed: 20452317]
23. Esteller M, Hamilton SR, Burger PC et al. (1999) Inactivation of the DNA repair gene 06-methylguanineDNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. Cancer Res 59:793–797 [PubMed: 10029064]
24. Verhaak RGW, Hoadley KA, Purdom E et al. (2010) An integrated genomic analysis identifies clinically relevant subtypes of glioblastoma characterized by abnormalities in PDGFRA, IDH1, EGFR and NF1. Cancer Cell 17(1):98–110 [PubMed: 20129251]
25. Phillips HS, Kharbanda S, Chen R et al. (2006) Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression and resemble stages in neurogenesis. Cancer Cell 9:157–173 [PubMed: 16530701]
26. Macdonald DR, Cascino TL, Schold SC Jr, Cairncross JG (1990) Response criteria for phase II studies of supratentorial malignant glioma. J Clin Oncol 8:1277–1280 [PubMed: 2358840]
27. Wong H, Alicke B, West KA et al. (2011) Pharmacokinetic-pharmacodynamic analysis of vismodegib in preclinical models of mutational and ligand-dependent Hedgehog pathway activation. Clin Cancer Res 17(14):4682–4692. 10.1158/1078-0432.CCR-11-0975 [PubMed: 21610148]
28. LoRusso PM, Rudin CM, Reddy JC et al. (2011) Phase I trial of Hedgehog pathway inhibitor GDC-0449 (GDC-0449) in patients with refractory, locally advanced or metastatic solid tumors. Clin Cancer Res 17(8):2052–2511
29. Chen J, Li Y, Yu TS et al. (2012) A restricted cell population propagates glioblastoma growth after chemotherapy. Nature 488(7412):522–526. 10.1038/nature11287 [PubMed: 22854781]
30. Domingo-Domenech J, Vidal SJ, Rodriguez-Bravo V et al. (2012) Suppression of acquired docetaxel resistance in prostate cancer through depletion of notch- and hedgehog-dependent tumor-initiating cells. Cancer Cell 22(3):373–388. 10.1016/j.ccr.2012.07.016 [PubMed: 22975379]
31. Stupp R, Mason WP, van den Bent MJ et al. (2005) Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352(10):987–996. 10.1056/NEJMoa043330 [PubMed: 15758009]
32. Nabors LB, Fiveash J (2005) Treatment of adults with recurrent malignant glioma. Expert Rev Neurother 5(4):509–514. 10.1586/14737175.5.4.509 [PubMed: 16026234]
33. Pitz MW, Desai A, Grossman SA, Blakeley JO (2011) Tissue concentration of systemically administered antineoplastic agents in human brain tumors. J Neuroncol 104(3):629–638. 10.1007/s11060-011-0564-y
34. Hoashi T, Kanda N, Saeki H (2022) Molecular mechanisms and targeted therapies of advanced basal cell carcinoma. Int J Mol Sci 23(19):11968. 10.3390/ijms231911968 [PubMed: 36233269]

Importance of the study

Effective treatment for recurrent glioblastoma (rGBM) remains an unmet clinical need with median survival typically ranging 5–6 months. There is significant evidence that GBM oncogenesis and treatment resistance is mediated by glioma stem cells (GSC), which have been demonstrated to be driven by various pathways including the sonic hedgehog (SHH) pathway. However, few clinical trials specifically targeting GSC have been performed and efficacy remains mostly untested. Here the authors present a phase O/II study targeting the hedgehog pathway in patients with rGBM. The treatment (GDC-0449; vismodegib) was well tolerated, reached the tumor, and inhibited $CD133^{+}$ neurosphere formation as well as SHH signaling, but had little clinical efficacy as a single agent in rGBM. This suggest that growth and maintenance of rGBM is not solely dependent on the SHH pathway and effective treatments targeting SMO may require combinations of therapeutic modalities.

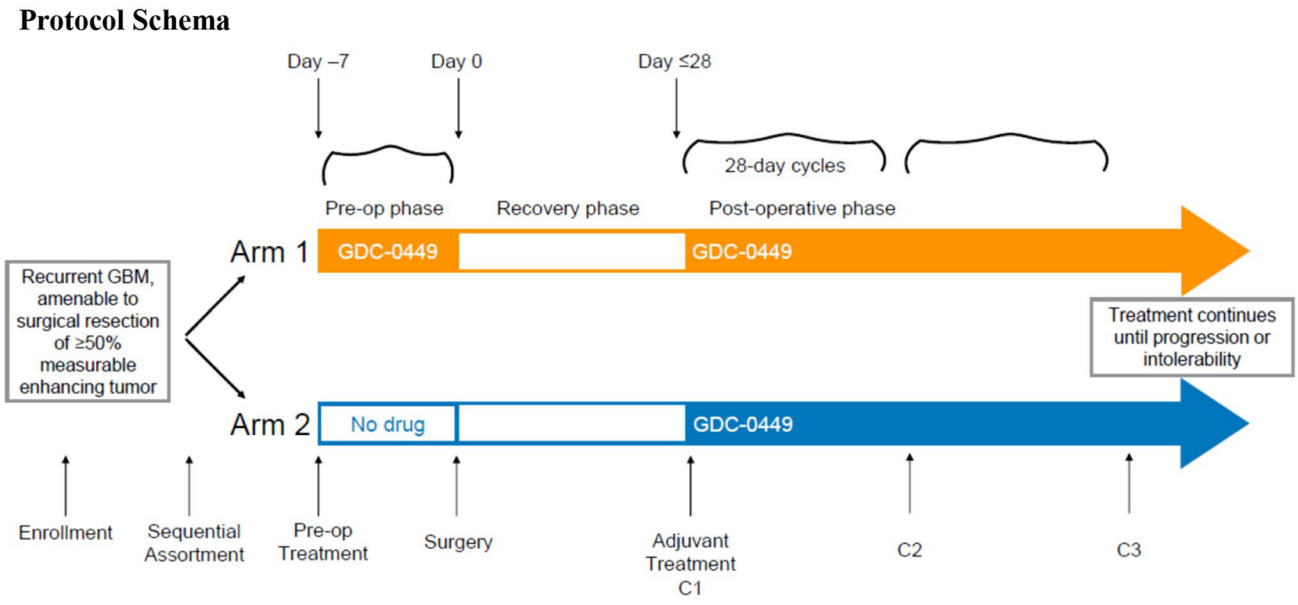

Fig. 1.
Clinical trial schema

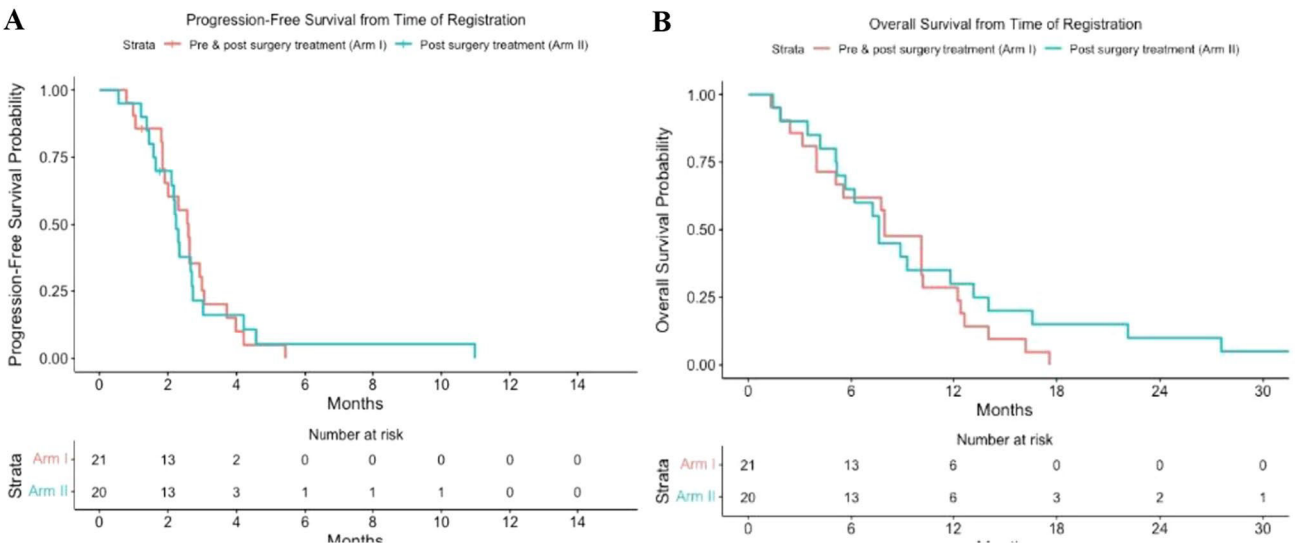

Fig. 2.

Progression Free & Overall Survival from Time to Registration by Arm. **A**PFS; **B** OS. There was no statistical difference in survival by arm (p = 0.98 and p = 0.37 respectively)

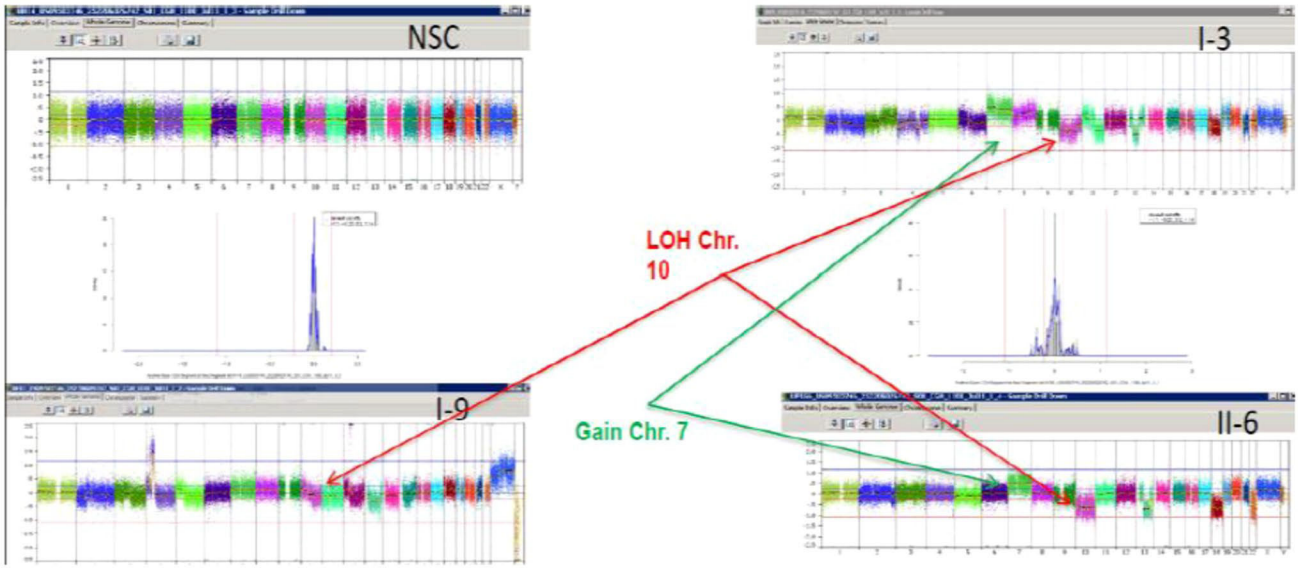

Fig. 3.

Representative Array comparative genomic hybridization (cGH) from patient derived NS and neural stem cells (NSC). Array cGH of tissue from neurospheres of representative patients in Arm I (3 and 9) as well as Arm II (5) are illustrated along with cells from neurospheres derived from NSC control as per methods. All three tumors demonstrated Gain of Chromosome 7 and LOH of chromosome 10 typical of many GBM. In contrast, no chromosomal abberations were noted in the neurospheres derived NSC

Table 1

Patient demographics

Characteristics	ARM I (n = 21)	ARM II (n = 20)	TOTAL N = 41
Age—year			
Median (range)	57.0 (32.9–79.2)	60.2(37.0–74.4)	58.2 (32.9–79.2)
Gender—No. (%)			
Male	9 (42.9)	10 (50)	19 (46.3)
Race– No. (%)			
White	20 (95.2)	20 (100)	40 (97.6)
KPS			
Median (range)	80 (60–100)	80 (60–90)	80 (60–100)
Mini Mental Score			
Median (range)	28 (16–30)	29 (16–30)	29 (16–30)
Prior No. of Relapses			
Median (range)	1 (1–4)	1 (1–2)	1 (1–4)
Prior No. of Surgical Procedure			
Median (range)	1 (1–3)	1 (1–4)	1 (1–4)
Steroid Usage No. (%)			
Yes	10 (47.6)	6 (30.0)	16 (39.0)
Histological Diagnosis No. (%)			
Glioblastoma	21 (100)	20 (100)	41 (100)

The demographics of patients in both arms were typical of patients in clinical trials for recurrent GBM.

There was no statistical difference between patients enrolled in the two arms

J Neurooncol. Author manuscript; available in PMC 2024 June 25.

Table 2

Toxicity in pre-surgical and post-surgical arms (arms I and II respectively)

2A. Toxicity in Arm I. No. of patients (%)	**Grade 1**	**Grade 2**	**Grade 3**	**Total**
Abdominal infection			1(5)	1(5)
Abdominal pain			1(5)	1(5)
Activated partial thromboplastin time prolonged	1(5)			1(5)
Alanine aminotransferase increased	4(19)			4(19)
Anemia	3(14)			3(14)
Anorexia	2(10)	2(10)		4(19)
Aspartate aminotransferase increased	1(5)			1(5)
Atrial flutter			1(5)	1(5)
Back pain	1(5)			1(5)
Constipation	2(10)			2(10)
Diarrhea	3(14)			3(14)
Dysgeusia	1(5)			1(5)
Facial muscle weakness		1(5)		1(5)
Fatigue	2(10)	5(24)		7(33)
Gastroesophageal reflux disease	1(5)			1(5)
Headache	1(5)			1(5)
Hypocalcemia	1(5)			1(5)
Hypokalemia	1(5)			1(5)
Insomnia	1(5)			1(5)
Lymphocyte count decreased	1(5)		1(5)	2(10)
Muscle weakness left-sided	2(10)			2 (10) (5)
Nausea	1(5)			1(5)
Neutrophil count decreased	1 (5) ()			1(5)
Platelet Count Decreased	3 (14)			3(14)
Pruritus	1(5)	1(5)		2(10)
Rash acneiform	1(5)			1(5)

Stroke			1(5)	1(5)
White Blood Cell Decreased	2(10)			2(10)
Toxicity in Arm II. No. of patients (%)	**Grade 1**	**Grade 2**	**Grade 3**	**Total**
Alopecia	1(5)	1(5)		2(10)
Anemia	4(20)			4(20)
Anorexia	3(15)	2(10)		5(25)
Back pain	1(5)			1(5)
Constipation	2(10)	1(5)		3(15)
Diarrhea	2(10)			2(10)
Dizziness	1(5)	1(5)		2(10)
Dry Skin	1(5)			1(5)
Dysgeusia	2(10)			2(10)
Dyspepsia		1(5)		1(5)
Fatigue	3(15)	4(20)		7(35)
Hand cramping	1(5)			1(5)
Headache	1(5)	2(10)		3(15)
Hematuria	1(5)			1(5)
Hyperglycemia	1(5)			1(5)
Hyperkalemia	1(5)			1(5)
Hypermagnesemia	1(5)			1(5)
Hypernatremia	4(20)			4(20)
Hypocalcemia	1(5)			1(5)
Hypoglycemia	1(5)			1(5)
Hypokalemia	2			2(10)
Hypophosphatemia			1(5)	
Insomnia		1(5)		1(5)
Joint cramping	1(5)			1(5)
Lymphocyte count decreased		1(5)		1(5)
Myalgia		1(5)		1(5)
Nausea	4(20)	1(5)		5(25)

Neutrophil count decreased	2(10)	1(5)	3(15)
Non-cardiac chest pain	1(5)		1(5)
Pain of skin	1(5)		1(5)
Platelet Count Decreased	1(5)		1(5)
Proteinuria	1(5)		1(5)
Rash acneiform		1(5)	1(5)
Urinary tract infection		1(5)	1(5)
Urine discoloration	1(5)		1(5)
White Blood Cell Decreased	4(20)		4(20)

Grade II fatigue was common in both arms (24% and 20% respectively) toxicity was otherwise mainly grade I with occasional (5–10%) grade II toxicity, and rare grade III toxicity. Grade IV toxicity was not observed