Cancer Biol Med 2024. doi: 10.20892/j.issn.2095-3941.2023.0373

ORIGINAL ARTICLE

OPEN ACCESS

Efficacy and safety of anlotinib combined with the STUPP regimen in patients with newly diagnosed glioblastoma: a multicenter, single-arm, phase II trial

Shuzhen Lai[1,2*], Peijing Li[3*], Xiaohui Liu[4], Guihong Liu[5], Tieming Xie[6], Xing Zhang[7], Xiaoxuan Wang[7], Jing Huang[8], Yiqiang Tang[9], Zhigang Liu[10,11], Guoping Shen[12], Chaoming Li[13], Fangxiao Lu[6], Lei Wang[3], Fagui Jiang[14], Caixing Sun[15], Yuanyuan Chen[16,17], Ming Chen[1,16,17]

[1]Department of Radiation Oncology, The Second Affiliated Hospital of Soochow University, Suzhou 215000, China; [2]Department of Radiation Oncology, Yuebei People's Hospital Affiliated to the Medical College of Shantou University, Shaoguan 512026, China; [3]Department of Radiation Oncology, The Cancer Hospital of the University of Chinese Academy of Sciences [Zhejiang Cancer Hospital Institute of Basic Medicine and Cancer (IBMC)], Chinese Academy of Sciences, Hangzhou 310022, China; [4]Department of Radiation Oncology, The Second Clinical Medical College of Zhejiang Chinese Medical University, Hangzhou 311403, China; [5]Department of Radiation Oncology, The Affiliated Hospital of Xuzhou Medical University, Xuzhou 221004, China; [6]Department of Radiology, The Cancer Hospital of the University of Chinese Academy of Sciences [Zhejiang Cancer Hospital Institute of Basic Medicine and Cancer (IBMC)], Chinese Academy of Sciences, Hangzhou 310022, China; [7]State Key Laboratory of Translational Medicine and Innovative Drug Development, Jiangsu Simcere Diagnostics Co., Ltd, Nanjing 210000, China; [8]Department of Radiation Oncology, The Affiliated Huai'an First People's Hospital of Nanjing Medical University, Huai'an 223399, China; [9]Department of Radiation Oncology, Jiangxi Cancer Hospital of Nanchang University, Nanchang 330029, China; [10]Department of Radiotherapy, Dongguan Institute of Clinical Cancer Research, Dongguan Key Laboratory of Precision Diagnosis and Treatment for Tumors, Affiliated Dongguan Hospital, Southern Medical University, Dongguan 523059, China; [11]The Cancer Center, The Fifth Affiliated Hospital of Sun Yat-Sen University, Zhuhai 519000, China; [12]Department of Radiation Oncology, The First Affiliated Hospital of Sun Yat-Sen University, Guangzhou 510080, China; [13]Department of Oncology, Maoming People's Hospital, Maoming 525000, China; [14]Department of Radiation Oncology, Kecheng District People's Hospital, Quzhou 324003, China; [15]Department of Neurosurgery, The Cancer Hospital of the University of Chinese Academy of Sciences [Zhejiang Cancer Hospital Institute of Basic Medicine and Cancer (IBMC)], Chinese Academy of Sciences, Hangzhou 310022, China; [16]Department of Radiation Oncology, State Key Laboratory of Oncology in South China, Sun Yat-sen University Cancer Center, Guangzhou 510060, China; [17]United Laboratory of Frontier Radiotherapy Technology of Sun Yat-sen University & Chinese Academy of Sciences Ion Medical Technology Co., Ltd, Guangzhou 510060, China

ABSTRACT

Objective: Glioblastomas are highly vascularized malignant tumors. We determined the efficacy and safety of the anti-angiogenic multi-kinase inhibitor, anlotinib, for a newly diagnosed glioblastoma.

Methods: This multicenter, single-arm trial (NCT04119674) enrolled 33 treatment-naïve patients with histologically proven glioblastomas between March 2019 and November 2020. Patients underwent treatment with the standard STUPP regimen [fractionated focal irradiation in daily fractions of 1.8-2 Gy given 5 d/w × 6 w (total = 54-60 Gy)] or radiotherapy plus continuous daily temozolomide (TMZ) (75 mg/m^2 of body surface area/d, 7 d/w from the first to the last day of radiotherapy), followed by 6 cycles of adjuvant TMZ (150–200 mg/m^2 × 5 d during each 28-d cycle) plus anlotinib (8 mg/d on d 1–14 of each 3-w cycle for 2 cycles during concomitant chemoradiotherapy, 8 maximal cycles as adjuvant therapy, followed by maintenance at 8 mg/d. The primary endpoint was progression-free survival (PFS). Secondary endpoints included overall survival (OS) and adverse events (AEs).

Results: Thirty-three patients received the planned treatment. The median PFS was 10.9 months (95% CI, 9.9–18.7 months) and the 12-month PFS rate was 48.5%. The median OS was 17.4 months (95% CI, 14.5–21.1 months) and the 12-month OS rate was 81.8%. The most common AEs included hypertriglyceridemia [58% (n = 19)], hypoalbuminemia [46% (n = 15)], and hypercholesterolemia [46% (n = 15)] during concurrent chemoradiotherapy and leukopenia [73% (n = 24)], hypertriglyceridemia [67% (n = 22)], and neutropenia [52% (n = 17)] during adjuvant therapy. Five patients discontinued treatment due to AEs. *HEG1* (HR, 5.6; 95% CI, 1.3–23.7; P = 0.021) and *RP1L1* alterations (HR, 11.1; 95% CI, 2.2–57.2; P = 0.004) were associated with a significantly shorter PFS.

Conclusions: Anlotinib plus the STUPP regimen has promising anti-tumor activity against glioblastoma and manageable toxicity. *HEG1* and *RP1L1* alterations might be novel predictive biomarkers of the response to anlotinib.

KEYWORDS Glioblastoma; anti-angiogenesis; multi-kinase inhibitor; anlotinib; temozolomide; progression-free survival

Introduction

Glioblastoma multiforme (GBM) is the most common aggressive intracranial tumor affecting adults, accounting for 48.6% of primary malignant brain tumors[1]. Despite our current understanding of the molecular mechanisms underlying GBM[2-4], using the Stupp protocol as the current standard regimen, which consists of maximal surgical debulking followed by radiotherapy with concurrent and subsequent temozolomide (TMZ) [fractionated focal irradiation in daily fractions of 2 Gy given 5 d/w × 6 w (total = 60 Gy)] or radiotherapy plus continuous daily TMZ (75 mg/m^2 of body surface area/d, 7 d/w from the first to the last day of radiotherapy), followed by 6 cycles of adjuvant TMZ (150–200 mg/m^2 × 5 d during each 28-d cycle), the prognosis of patients with GBM remains dismal, with a median progression-free survival (PFS) of 6.9 months and an overall survival (OS) of 14.6 months[5]. A recent retrospective study showed that GBM patient outcome has not improved over the last decade with the median OS < 16 months, even in the subgroup with an excellent prognosis[2], highlighting the urgent need for novel effective and safe therapeutic regimens.

GBM is highly invasive and marked for rampant genomic instability. Robust aberrant angiogenesis renders GBM potentially amenable to anti-angiogenic therapy[6,7]. Nevertheless, bevacizumab, an anti-VEGF antibody, failed to significantly extend the OS of newly diagnosed GBM patients when added to the standard of care (SOC) in the first-line setting, and only prolonged the PFS by 3–4 months[8,9]. An important reason for bevacizumab failing to improve the OS is that bevacizumab only targets a single signaling angiogenesis pathway[10], highlighting the importance of simultaneously targeting multiple pro-angiogenic growth factors. Although attempts have been made to treat GBM with anti-angiogenic multi-kinase inhibitors (MKIs), such as vandetanib and sorafenib, these drugs likely failed to improve prognosis due to inadequate blood–brain barrier (BBB) penetration, limited VEGFR2 and/or EGFR inhibition, or intolerable toxicities[11,12].

Anlotinib, a novel MKI, targets onco-angiogenesis and suppresses tumor growth by simultaneously blocking VEGFR, FGFR, PDGFRA, and c-Kit, therefore exerting a broad spectrum of tumor inhibitory effects[13]. Anlotinib has been approved as a 2nd-line agent in China for advanced soft tissue sarcomas and advanced non-small cell lung cancer (NSCLC). Anlotinib has also been investigated for other advanced solid tumors[14]. Anlotinib has been shown to have activity in patients with brain metastases from NSCLC, delaying the time to brain progression[15]. Our preclinical study showed that radiation renders the BBB more permeable to anlotinib[16]. Together, anlotinib and radiation were shown to be synergistic in suppressing orthotopic glioma growth[16], suggesting that anlotinib might be active against intracranial tumors. However, no prospective study has involved anlotinib as monotherapy or in combination with TMZ for newly diagnosed GBM.

In this phase II trial we investigated the efficacy and safety of anlotinib added to the current SOC for newly diagnosed GBM. Because no effective predictive biomarkers have been identified that enable biomarker-stratified anti-angiogenic therapies[17], we further performed an exploratory analysis of genomic profiles in this cohort to identify potential predictors of treatment response using whole exome sequencing (WES).

Materials and methods

Study population

We conducted this multicenter, single-arm, phase II trial at seven centers in China. Patients 18–70 years of age with histologically proven GBM based on the 2016 World Health Organization (WHO) classification of central nervous system (CNS) tumors were eligible for the study[18]. The patients had adequate organ function and a Karnofsky Performance Status (KPS) score ≥ 60. Patients had to have at least one measurable lesion according to the Response Assessment in Neuro-Oncology (RANO) criteria and received no prior radiotherapy, chemotherapy, immunotherapy, or biologic therapy. Other eligibility criteria are described in the submitted study protocol.

The trial was conducted per the provisions of the Declaration of Helsinki and the International Conference on Harmonisation guidelines for Good Clinical Practice. The trial protocol was approved by the Cancer Hospital Institutional Review Board (IRB) of the University of Chinese Academy of Sciences

*These authors contributed equally to this work.
Correspondence to: Yuanyuan Chen and Ming Chen
E-mail: chenyy2@sysucc.org.cn and chenming@sysucc.org.cn
ORCID ID: https://orcid.org/0000-0002-9655-7828
and https://orcid.org/0000-0003-2570-0858
Received October 14, 2023; accepted January 29, 2023;
published online March 4, 2024.
Available at www.cancerbiomed.org

[Zhejiang Cancer Hospital (IRB-2018-238)]. All patients provided written informed consent before enrollment. The trial is registered with CLINICALTRIALS.GOV (NCT04119674). The study protocol adhered to the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) statement and the study report adhered to the Consolidated Standards of Reporting Trials (CONSORT) statement.

Treatments

Radiotherapy was initiated 4–6 weeks postoperatively at a dose of 1.8–2.0 Grays (Gy)/fraction 5 d/w × 6 w for a total dose of 54–60 Gy. TMZ (75 mg/m^2/d) was administered for a maximum of 49 d. Beginning 4 w after completion of concurrent chemoradiotherapy, patients received adjuvant TMZ for 5 d every 28 d; the first cycle was 150 mg/m^2/d and subsequent cycles were 200 mg/m^2/d for a maximum of 6 cycles (**Supplementary Figure S1A**). The TMZ dose was adjusted upon occurrences of unacceptable toxicities. Chemoradiotherapy was delayed a maximum of 2 w.

Anlotinib (8 mg/d; Chia Tai Tianqing Pharmaceutical Group Co., Ltd., China) was administered orally on d 1–14 of each 3-week cycle × 2 cycles during concomitant therapy for a maximum of 8 cycles during adjuvant chemotherapy. One week after discontinuation of adjuvant chemotherapy, anlotinib (8 mg/d) was given for maintenance (**Supplementary Figure S1B**). Treatment was continued until progressive disease (PD) per Response Assessment in Neuro-Oncology (RANO). Anlotinib dose reduction was not permitted but a maximum delay of 2 w was allowed for recovery from toxicity.

Patient assessment

The baseline evaluation included full clinical and neurologic evaluations, brain magnetic resonance imaging (MRI), electrocardiography, complete blood count, blood chemical analyses, and urinalysis. Isocitrate dehydrogenase-1 (*IDH*) mutation and methylguanine-DNA methyltransferase gene (*MGMT*) promoter methylation status was recorded based on the surgical pathology reports. During concurrent chemoradiation therapy, patients received weekly clinical assessment and laboratory evaluations. Clinical assessment was also performed 1 w before and 3 and 7 d after the end of each adjuvant cycle of TMZ and 1 w before the start of each cycle of anlotinib. Patients had follow-up evaluations every 2 months during the first year and every 3 months thereafter. Radiologic assessments were performed on d 21 and 42 of concurrent chemoradiation therapy, 1 w prior to the start of adjuvant cycle 1, 3, and 5, every 3 cycles of anlotinib, and during follow-up evaluations per RANO criteria. Adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events [NCI CTCAE (version 4.0)].

WES

Genomic DNA was extracted from formalin-fixed paraffin-embedded tumor samples with > 20% tumor cells and matched blood samples. The library was constructed using the KAPA Library Preparation kit (Roche Diagnostics, Rotkreuz, Switzerland). Sequencing was performed with an average depth of 500X for the whole exome using the Illumina Novaseq 6000 system (San Diego, CA, USA). A custom pipeline was established, including reads alignment, variants calling, identification of copy number variations (CNVs), and fusions, as well as quality control. The adapter-trimming was conducted using fastp (v.2.20.0, https://github.com/OpenGene/fastp) and cleaned paired-end reads in FASTQ format were aligned to the human reference genome (hg19) with BWA-mem (v.0.7.17, https://github.com/lh3/bwa), among which selected regions were realigned with ABRA2 (v2.21, https://github.com/mozack/abra2). Single nucleotide variants and short insertion and deletion variants were both called using VarDict (v.1.5.7, https://github.com/AstraZeneca-NGS/VarDict) and InterVar (https://github.com/WGLab/InterVar)[19,20]. CNVkit (v.0.9.10, https://github.com/etal/cnvkit) and FACTERA (v1.4.4, https://factera.stanford.edu/) were utilized to identify CNVs and fusions, respectively[21,22]. Additionally, custom scripts were implemented for analyzing mutational filters and inspection.

Mutation calling

To ensure high-quality mutation calls, the following filtering criteria were applied: 1) sequencing depth ≥ 20 × in tumor DNA and ≥ 10 ×in germline DNA; 2) variant allele frequency ≥ 0.02 in tumor DNA and < 0.01 in germline DNA; 3) the total number of reads supporting the variant calls was ≥ 4; 4) variant frequency was < 0.01 in ESP6500, 1000 genome, and EXAC databases; and 5) logarithm of odds (LOD) score > 18 (MuTect default was 6.3). We kept the mutations that passed all filtering criteria, except an LOD score < 18, if the identical mutations were present with an LOD score ≥ 18 in other

regions within the same tumors. Cancer gene mutations were defined as identical oncogene mutations previously reported, stop gains and frameshift of tumor suppressor genes, and other non-synonymous mutations with a Combined Annotation Dependent Depletion (CADD) score > 20.

Outcomes

The primary endpoint was the PFS, which was calculated from the date of study entry to progressive disease (PD) or death, whichever occurred first. Secondary endpoints included the OS, which was calculated from the date of study entry to death from all causes, treatment failures, and occurrences of treatment-related adverse events (TRAEs). Exploratory outcomes were the association between genetic variants and relapse, PFS, OS, or TRAEs.

Statistical analysis

Assuming a median PFS of 6.9 months based on historical controls, a power of 80%, and a one-sided α error of 10%[5], a sample size of 31 patients was required to detect a 3.7-month-increase to reach a median PFS of 10.6 months[9]. Assuming a dropout rate of 5%, an accrual size of 33 patients was chosen in this trial.

Statistical analyses were pre-specified and all enrollees were evaluated for efficacy measures and safety. Recursive partitioning analysis (RPA) class was determined using age, KPS at the time of treatment, extent of resection, and mental status[23]. The differences between clinical and mutational frequencies were calculated using a two-sided χ^2 or Fisher's exact test, as indicated. The odds ratio (OR) with a 95% confidence interval (CI) was provided. The Wilcoxon rank-sum test was performed to compare the distribution differences between the two groups. The follow-up duration was calculated using the reverse Kaplan-Meier method. Survival curves were estimated with the Kaplan-Meier method and compared using the log-rank or Tarone-Ware test. Univariate and multivariate Cox regression was used to analyze the influence of genetic mutations on prognosis and clinicopathologic factors that had satisfied the proportional hazards assumption. Spearman correlation was performed to test the association between GBM gene expression in The Cancer Genome Atlas (TCGA) database.

All statistical analyses and graphics used software R (v4.0.2). A $P < 0.05$ was considered significant for all hypotheses tested.

Results

Patient characteristics

Between March 2019 and November 2020, 36 patients were screened and 33 were eligible and received the allocated study interventions (**Supplementary Figure S1C**). The median patient age was 52 years (range, 52–69 years), 52% were males, and the median KPS score was 90. Thirty percent of the patients had undergone a gross total tumor resection and 52% had a subtotal tumor resection. Two patients (6%) had an IDH1 mutation (R132H) and 11 had [11/31 (36%)] had *MGMT* promoter methylation (**Table 1**).

Treatment compliance

All patients completed scheduled radiotherapy and concomitant chemotherapy. There were no treatment interruptions for anlotinib and no dose reduction for TMZ due to AEs. Twenty-nine patients (88%) completed adjuvant TMZ. Four patients (12%) discontinued adjuvant TMZ due to PD; 2 of the 4 patients completed 5 cycles of anlotinib and the remaining 2 patients received 6 and 7 cycles. Four patients did not receive maintenance therapy with anlotinib due to PD and 1 patient discontinued due to AEs after completion of adjuvant treatment. Twenty-four patients received maintenance therapy with anlotinib. Eighteen patients (55%) discontinued treatment because of PD and 5 (15%) discontinued treatment due to AEs. Ten patients (30%) were still receiving treatment on the data cut-off date (August 31, 2022). The median number of anlotinib cycles was 13 (range, 7–47).

Survival outcomes

The median follow-up duration was 25.2 months (95% CI, 20.9–29.5 months). Twenty-three PFS events had occurred and the median PFS was 10.9 months (95% CI, 9.9–18.7 months; **Figure 1A**) by the data cut-off date. The 6- and 12-month PFS rate was 97.0% and 48.5%, respectively. The median OS was 17.4 months (95% CI, 14.5–21.1 months) and the 6-, 12- and 18-month OS rates were 100%, 81.8%, and 48.5%, respectively (**Figure 1B**).

Survival analysis of clinical factors based on Cox regression and Kaplan-Meier analyses are shown in **Figure 1C, D** and **Supplementary Figure S2A-F**. The median PFS was not

Table 1 Demographic and baseline characteristics of the patients and those detected by WES

	Patients included in the trial (*n* = 33)	Patients included in the WES analysis (*n* = 21)
Characteristic		
Age, years		
Median	52	54
Range	32–69	32–68
Gender		
Male	17 (52)	11 (52)
Female	16 (49)	10 (48)
KPS score		
Median	90	90
≥ 90	22 (67)	15 (71)
60–80	11 (33)	6 (29)
RPA class		
III	7 (21)	3 (14)
IV	13 (39)	10 (48)
V	13 (39)	8 (38)
Co-morbidities		
No	25 (76)	17 (81)
Yes	8 (24)	4 (19)
Hypertension and diabetes	2 (6)	2 (10)
Hypertension	6 (18)	2 (10)
BMI, kg/m^2		
< 18.5	2 (6)	2 (10)
18.5 ≤ BMI < 24	15 (45)	9 (43)
≥ 24	16 (49)	10 (48)
Extent of surgical resection		
Gross total resection	10 (30)	7 (33)
Near-total resection	6 (18)	4 (19)
Subtotal resection	17 (52)	10 (18)
Ki-67 index		
≤ 30%	15 (45)	11 (52)
> 30%	13 (39)	7 (33)
Unknown	5 (15)	3 (14)
MGMT promotor methylation status		
Methylated	11 (33)	11 (52)
Unmethylated	20 (61)	8 (38)
Undetermined	2 (6)	2 (10)
IDH1/2 mutation status		
Mutant	2 (6)	2 (10)
Wild-type	31 (94)	19 (91)

BMI, body mass index; IDH, isocitrate dehydrogenase; KPS, Karnofsky performance status; MGMT, methylguanine-DNA methyltransferase; RPA, recursive partitioning analysis; WES, whole exome sequencing. Data are expressed as the number (%) unless otherwise specified. *IDH*1/2 mutation and *MGMT* promoter methylation status are based on the surgical pathology reports.

reached in patients with a methylated *MGMT* promoter and was significantly longer than patients with an unmethylated *MGMT* promoter (10.1 months; 95% CI, 8.6–14.8 months; HR, 0.36; 95% CI, 0.13–0.98; $P = 0.047$). No significant difference was detected in the median PFS between patients with a Ki-67 ≤ 30% and those with a Ki-67 > 30% (HR, 1.7; 95% CI, 0.68–4.2; $P = 0.26$). No significant difference was detected in the median OS between patients with and without an *MGMT* promoter methylation (HR, 0.54; 95% CI, 0.19–1.5; $P = 0.23$), and patients with a Ki-67 ≤ 30% and those with a Ki-67 > 30% (HR, 2.3; 95% CI, 0.84–6.4; $P = 0.1$).

In addition, the log-rank and Tarone-Ware tests showed no center effects for PFS ($P = 0.21$ and 0.15, respectively) and OS ($P = 0.16$ and 0.19, respectively).

Genomic alterations and treatment response

A subset of 21 patients whose tumors carried genomic alterations, as determined by WES, were included in the secondary analysis (**Table 1**). The most frequently altered gene was *TERT* (81%), followed by *CDK6* (52%), *TP53* (48%), *CDKN2A/B* (43%), and *EGFR* (43%; **Figure 2A**). *PIK3CA*, *HEG1*, *HMCN1* and *RP1L1* mutations only occurred in GBM patients who relapsed. In contrast, *MYO15A* and *ATRX* mutations only occurred in patients who had not relapsed (**Figure 2B**).

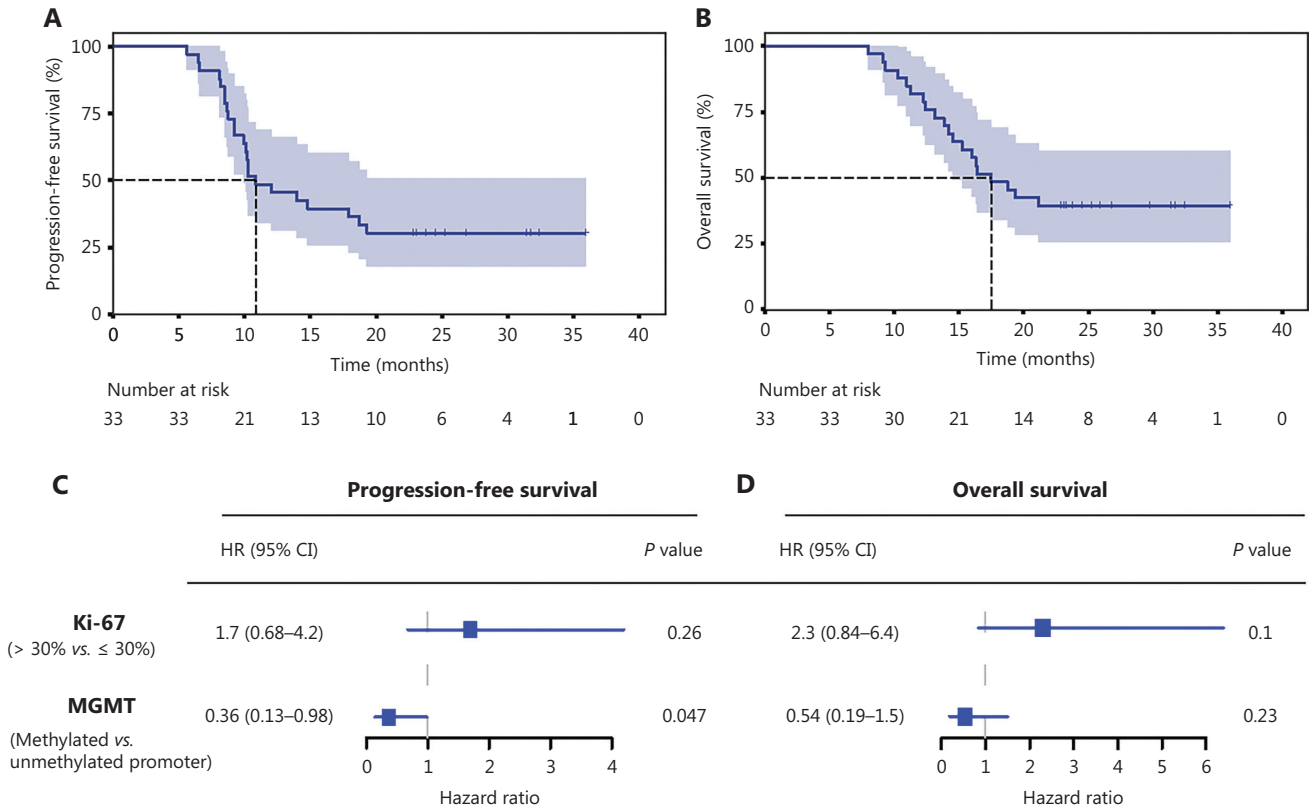

Figure 1 Survival outcomes of the study patients. The Kaplan-Meier curves of progression-free survival (PFS) (A) and overall survival (OS) (B). Forest plot of PFS (C) and OS (D) per patient subgroup.

Multivariate Cox regression revealed that both *RP1L1* (HR, 12.5; 95% CI, 2.1–74.3; $P = 0.005$) and *HEG1* alterations (HR, 6.0; 95% CI, 1.2–28.8; $P = 0.026$) were significant and independent adverse predictors of PFS (**Table 2** and **Figure 2C, D**). Furthermore, *RP1L1* mutations were a significant and independent adverse predictor of OS (HR, 33.4; 95% CI, 3.2–351.4; $P = 0.003$; **Table 2** and **Figure 2E**), while mutated *HEG1* was not a significant determinant of OS (HR, 2.5; 95% CI, 0.6–10.1; $P = 0.210$; **Figure 2F**).

RP1L1 expression was significantly correlated with angiogenesis-related genes, including *PDGFRA* ($r = 0.27$; $P < 0.001$), *FLT1* ($r = 0.50$; $P < 0.001$), *KDR* ($r = 0.44$; $P < 0.001$), *KIT* ($r = 0.30$; $P < 0.001$), *FGFR1* ($r = 0.35$; $P < 0.001$), and *FGFR3* ($r = 0.32$; $P < 0.001$; **Supplementary Figure S3A**). *HEG1* expression was significantly correlated with *FLT1* ($r = 0.55$; $P < 0.001$), *KDR* ($r = 0.57$; $P < 0.001$), *MET* ($r = 0.19$; $P = 0.016$), *KIT* ($r = 0.29$; $P < 0.001$), *FGFR1* ($r = 0.42$; $P < 0.001$), *FGFR2* ($r = 0.21$; $P = 0.007$), and *FGFR3* ($r = 0.34$; $P < 0.001$; **Supplementary Figure S3B**). The relapse and non-relapse groups had no significant differences with respect to TMB treatment, mutational frequency, and maximal allele mutational frequency ($P > 0.05$; **Supplementary Figure S4**).

Furthermore, alterations in the signaling pathways, including RTK, PI3K, RAS, RB/p53, and DNA damage response-associated proteins, did not predict PFS or OS (**Supplementary Table S1**).

Safety

Thirty-three patients received anlotinib plus SOC and were included in the safety analysis. The 3 most frequent treatment-emergent AEs (TEAEs) during concurrent chemoradiotherapy plus anlotinib were hypertriglyceridemia (58%), hypercholesterolemia (46%), and hypoalbuminemia (46%), while the 3 most common TEAEs during adjuvant chemotherapy plus anlotinib were leukopenia (73%), hypertriglyceridemia (67%), and neutropenia (52%). Two patients receiving concurrent chemoradiotherapy plus anlotinib developed grade 3 hypertriglyceridemia. One patient with grade 4 and 2 patients with grade 3 thrombocytopenia, 1 patient with grade

3 leukopenia, and 1 patient with grade 3 hypertriglyceridemia were reported during adjuvant therapy (**Table 3**).

Six patients (18%) required hospitalization due to AEs, including 1 with grade 1 seizures and 5 with grade 2 cerebral ischemia during maintenance therapy. Two patients with cerebral ischemia resumed treatment after appropriate management and 3 discontinued treatment. Interestingly, *PIK3CA* mutations were significantly associated with cerebral ischemia ($P = 0.032$; OR = 16.4); specifically, 3 patients with grade 2 cerebral ischemia who underwent WES all harbored *PIK3CA* mutations (**Supplementary Table S2**). Meanwhile, duration of anlotinib treatment was not related to cerebral ischemia ($P = 0.63$). Grade 3 body weight reduction occurred in 1 patient at the end of cycle 8 of maintenance therapy and one patient developed grade 4 thrombocytopenia during adjuvant therapy and discontinued treatment. No death was reported.

Discussion

We hypothesized that a MKI simultaneously targeting multiple pro-angiogenic factors with broad tumor inhibitory activities could improve the outcome of newly diagnosed GBM patients receiving SOC. In this multicenter, single-arm, phase

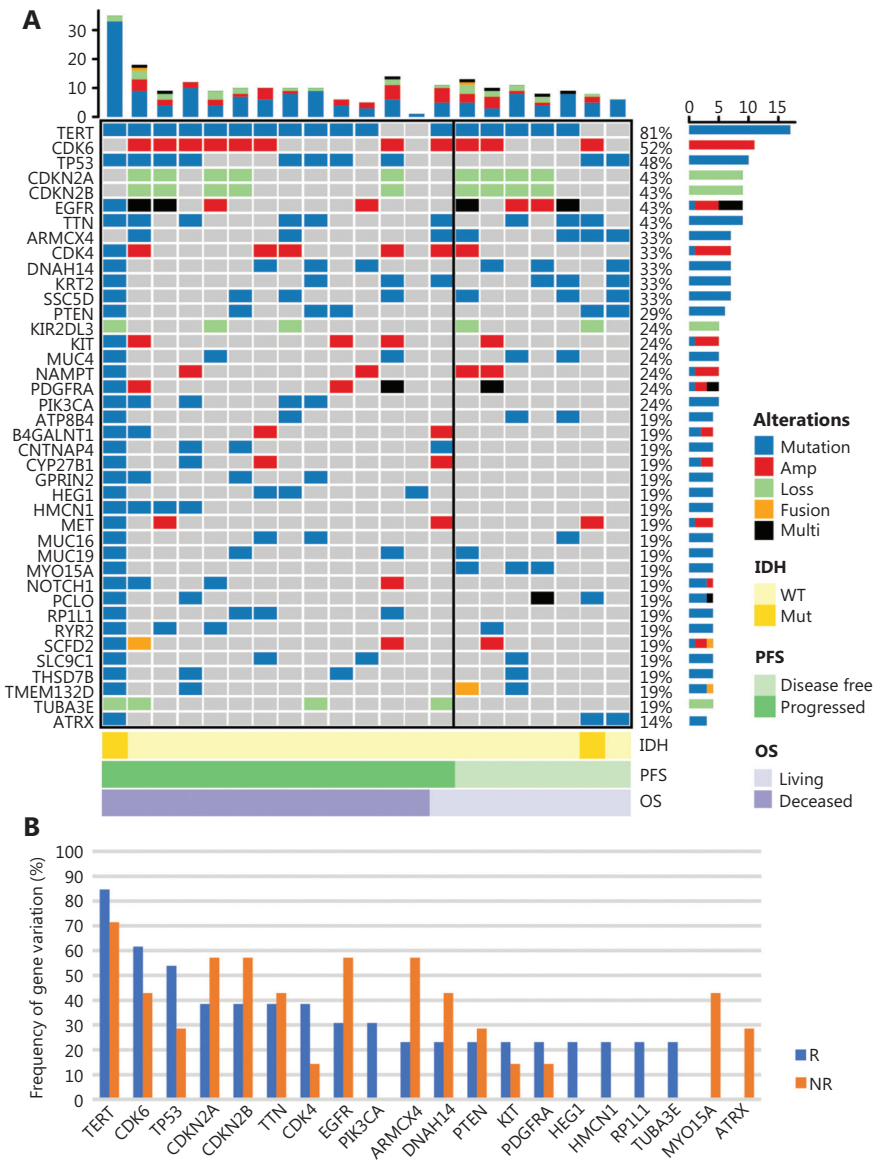

Figure 2 Continued

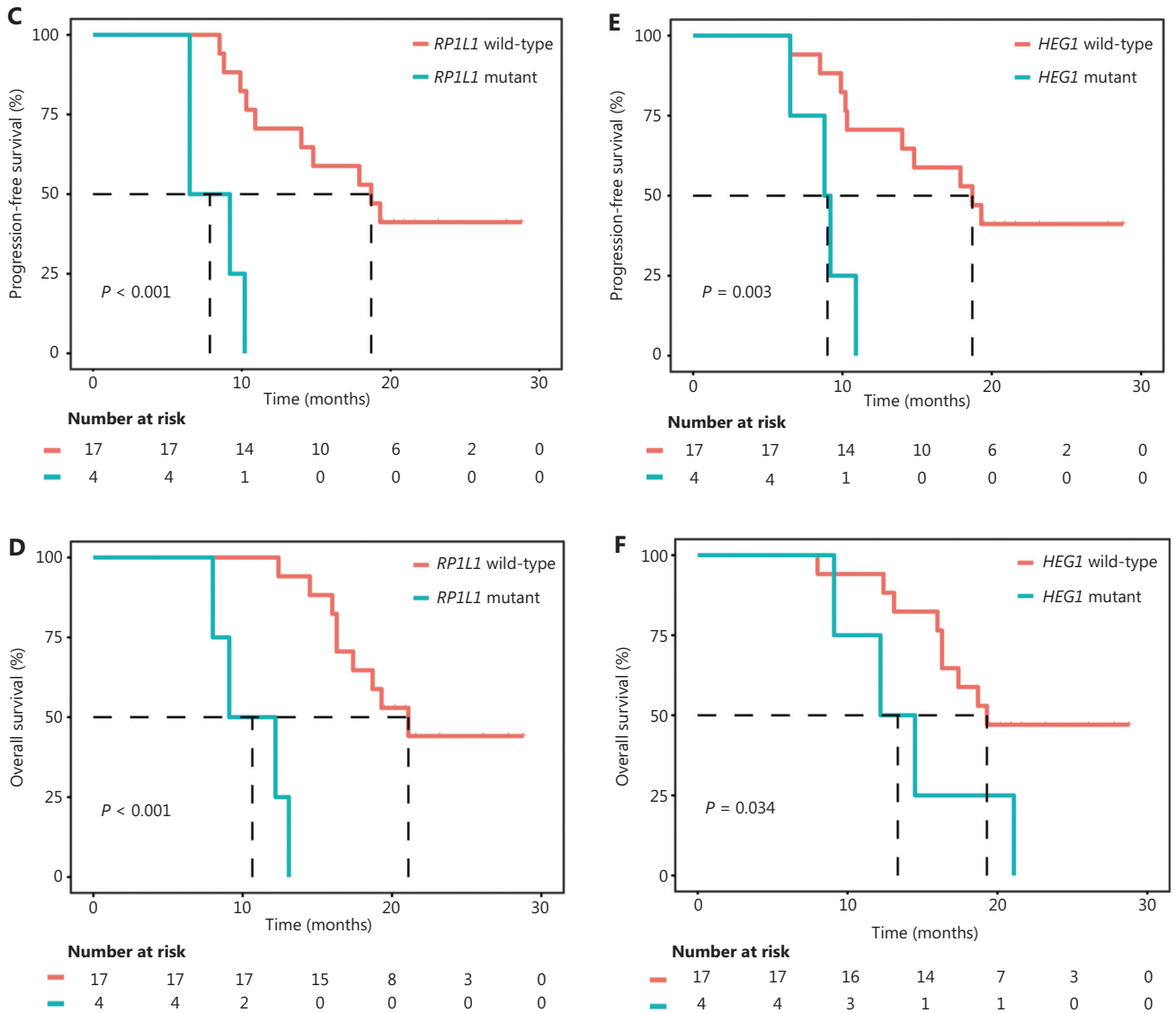

Figure 2 Distribution of genomic alterations with regard to response to treatment and survival analysis based on Kaplan-Meier curve in GBM patients in secondary analysis. (A) Mutational landscape of patients with glioblastoma multiforme (GBM) detected by whole exome sequencing (WES). (B) The frequency of gene variants between relapse and non-relapse patients. PFS in patients harboring *RP1L1* (C) and *HEG1* (D) alterations and wild-type. OS in patients with harboring *RP1L1* (E) and *HEG1* (F) alterations and wild-type.

II trial, newly diagnosed GBM patients attained a median PFS of 10.9 months (95% CI, 9.9–18.7 months), meeting the primary endpoint of the study, with a 12-month PFS of 48.5%. Furthermore, the median OS reached 17.4 months and the probabilities of survival at 12 months were 81.8%. The safety profile for anlotinib is acceptable and consistent with that observed in other tumors[14].

This study was the first trial to evaluate the efficacy and safety of anlotinib added to the SOC for first-line treatment of GBM, attaining a median PFS of 10.9 months, which is higher than that of historical controls (6.9 months)[5]. The PFS in our cohort is comparable to bevacizumab added to SOC for newly diagnosed GBM in RTOG-0825 (10.7 months) and AVAglio (10.6 months). PFS improvement with bevacizumab was significant *versus* placebo in AVAglio but did not reach the pre-specified threshold for significance in RTOG-0825 ($P < 0.004$)[8,9]. It is worth noting that progression assessment using the RANO criteria in our study was

Table 2 Univariate and multivariate Cox models for PFS and OS in the secondary analysis

Variables	Progression-free survival				Overall survival			
	Univariate analysis		Multivariate analysis		Univariate analysis		Multivariate analysis	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Age (> 54 *vs.* ≤ 54), years	1.1 (0.4–3.2)	0.873	0.5 (0.1–3.9)	0.546	1.6 (0.5–5.2)	0.399	0.3 (0.0–3.6)	0.365
Gender (female *vs.* male)	0.8 (0.3–2.3)	0.635	1.7 (0.5–5.8)	0.428	1.1 (0.4–3.5)	0.827	2.6 (0.6–10.1)	0.184
KPS (≥ 90 *vs.* 60–80)	1.2 (0.4–3.7)	0.810	0.8 (0.2–4.7)	0.849	1.6 (0.5–5.3)	0.458	1.4 (0.1–12.5)	0.792
Resection		0.787		0.500		0.414		0.140
NTR *vs.* GTR	1.2 (0.3–5.4)	0.796	2.1 (0.4–10.8)	0.396	2.2 (0.4–10.9)	0.357	5.1 (0.7–34.9)	0.330
STR *vs.* GTR	1.6 (0.4–5.5)	0.495	2.6 (0.5–13.9)	0.250	2.4 (0.6–9.7)	0.218	5.9 (0.9–38.5)	0.681
HEG1 (mutant *vs.* wild-type)	5.6 (1.3–23.7)	0.021	6.0 (1.2–28.8)	0.026	2.8 (0.7–10.3)	0.132	2.5 (0.6–10.1)	0.210
RP1L1 (mutant *vs.* wild-type)	11.1 (2.2–57.2)	0.004	12.5 (2.1–74.3)	0.005	33.8 (3.4–334.5)	0.003	33.4 (3.2–351.4)	0.003

GTR, gross total resection; KPS, Karnofsky performance status; NTR, near-total resection; OS, overall survival; PFS, progression-free survival. One hypermutator case with tumor mutational burden (TMB) = 327.7 was excluded from analysis.

Table 3 Summary of adverse events

Treatment-emergent AEs	Concomitant therapy		Adjuvant therapy	
	Any grade	Grade 3–4	Any grade	Grade 3–4
Hypertriglyceridemia	19 (58)	2 (6)	22 (67)	1 (3)
Hypercholesterolemia	15 (46)	0 (0)	9 (27)	0 (0)
Hypoalbuminemia	15 (46)	0 (0)	7 (21)	0 (0)
Leukopenia	11 (33)	0 (0)	24 (73)	1 (3)
Elevated g-glutamyl transferase	11 (33)	0 (0)	11 (33)	0 (0)
Neutropenia	10 (30)	0 (0)	17 (52)	0 (0)
Elevated aspartate transaminase	8 (24)	0 (0)	4 (12)	0 (0)
Elevated alanine aminotransferase	6 (18)	0 (0)	6 (18)	0 (0)
Hypertension	5 (15)	0 (0)	5 (15)	0 (0)
Fatigue	5 (15)	0 (0)	15 (46)	0 (0)
Thrombocytopenia	4 (12)	0 (0)	9 (27)	3 (6)
Nausea	4 (12)	0 (0)	6 (18)	0 (0)
Vomiting	4 (12)	0 (0)	5 (15)	0 (0)
Proteinuria	1 (3)	0 (0)	14 (42)	0 (0)
Seizure	1 (3)	0 (0)	4 (12)	0 (0)
Hypothyroidism	0 (0)	0 (0)	10 (30)	0 (0)
Palmar–plantar erythrodysaesthesia syndrome	0 (0)	0 (0)	4 (12.1)	0 (0)

Data are expressed as a number (%).

more accurate and efficient compared to previous studies using Macdonald criteria, which do not consider T2/FLAIR MRI results[8,9]. Another strength of the current study was that anlotinib offers a convenient oral dosing regimen without the need for additional infusions or admissions, which would not influence patient compliance.

The median OS was 17.4 months in this trial, 16.8 months in AVAglio, and 15.7 months in RTOG-0825. The OS in all 3 trials was longer than the Stupp trial (14.6 months)[5]. However, bevacizumab failed to improve the OS rates *versus* placebo in AVAglio and RTOG-0825. A retrospective analysis of the AVAglio data suggested that subgroups with *IDH1* wild-type pro-neural glioblastoma derived an OS benefit from bevacizumab, suggesting that the high tumor heterogeneity of GBM leads to different treatment responses. In our study *MGMT* methylation status was significantly associated with PFS, which is consistent with RTOG-0825. In contrast, the OS of patients with methylated *MGMT* promoter was not longer than patients with unmethylated *MGMT* promoter in AVAglio. *MGMT* methylation status is a predictor of TMZ resistance and a strong prognostic biomarker of newly diagnosed GBM regardless of treatment[24]. Our study did not identify subgroups of newly diagnosed GBM patients who achieved an OS or PFS benefit by the addition of anlotinib, including *MGMT* status, Ki-67, and co-morbidities. Future investigations involving predictive imaging markers and biomarkers that could stratify patients for anti-angiogenic therapy added to the SOC in newly diagnosed GBM patients are warranted.

A variety of methods have been used to study the biomarkers related to treatment response or drug resistance and to develop individualized precision treatment strategies[3,4]. WES based on next-generation sequencing (NGS) has been proven remarkably powerful in genomic analysis and is appropriate for both screening previously discovered and novel significant alterations[25]. In the secondary analysis both *HEG1* and *RP1L1* variants were independent biomarkers of PFS, and the latter variant was significantly associated with OS. Although none of the prognostic biomarkers of anti-angiogenic therapy have been previously proven, newly diagnosed GBM with a pro-neural gene expression signature was identified as a subgroup with better OS compared to placebo[26]. HEG1 has a critical role in angiogenesis, which is related to tumor progression. An HEG1 defect may result in deficiency of vessel formation, leading to a worse survival outcome of patients with less vascularized GBMs who receive anlotinib therapy[27]. *RP1L1* is a component of photoreceptor cilium, which is involved in cancer by association with hedgehog, Wnt, and PDGF signaling, and is pivotal in regulating the fate of glioma stem cells[28,29]. The effect and potential predictive values of *HEG1* and *RP1L1* alterations in response to anti-angiogenesis therapy of GBM should be the focus of corollary studies.

Overall, the study regimen was tolerable. There were no increased or unanticipated toxicities. Palmar–plantar erythrodysesthesia syndrome, a frequent AE of anlotinib[30], occurred in 4 patients (12%) during adjuvant treatment, but none were grade 3 or higher. Hypothyroidism, a common AE of MKI[31], occurred in 30% of the patients receiving adjuvant TMZ plus anlotinib, but none were grade 3 or higher. Hypertension, another common AE associated with angiogenesis inhibitors[32], occurred in 4 patients (12%) during concurrent chemoradiotherapy and 5 (15%) during adjuvant therapy. Bleeding, a major concern with anti-angiogenic therapy, was not reported in this study. However, grade 2 cerebral ischemia occurred in 5 patients during maintenance therapy, 2 of whom resumed treatment after appropriate management. Cerebrovascular ischemia occurred in 9% of newly diagnosed GBM patients receiving bevacizumab added to concurrent chemoradiotherapy and adjuvant TMZ in a phase II trial, which may arise from radiation-induced occlusive arteriopathy potentiated by bevacizumab[33]. Lipid abnormalities are another possible contributor to cerebrovascular ischemia. In the current study lipid abnormalities were observed during both concurrent chemoradiotherapy plus anlotinib [hypercholesterolemia (46%) and hypertriglyceridemia (58%)] and adjuvant chemotherapy plus anlotinib [hypertriglyceridemia (67%)] and should be carefully monitored because of the association with arterial thromboembolic events. Interestingly, *PIK3CA* mutations were significantly associated with cerebrovascular ischemia in the secondary analysis but ischemia did not increase with prolongation of treatment. The overall acceptable toxicity profile of the study regimen may be partially due to the low dose (8 mg) of anlotinib, which is typically given at 10 or 12 mg in other tumors.

The current study had several limitations. This was a single arm study without a control group and the sample size was limited. Based on the encouraging results of our preclinical study and the current trial[16], a randomized controlled study (NCT04959500) is ongoing that includes a larger sample size. In addition, the current study did not evaluate changes in neurocognitive function and quality of life among the patients in the cohort. In the current study a decline in KPS scores was only noted in patients during concurrent radiochemotherapy. No KPS decline due to AEs was observed.

Conclusions

In conclusion, anlotinib added to the current SOC for newly diagnosed GBM exhibited encouraging anti-tumor activities

and had an acceptable toxicity profile. *HEG1* and *RP1L1* alterations could be novel predictive biomarkers in patients with newly diagnosed GBM treated with anlotinib plus SOC. Future randomized controlled trials and application of biomarkers are warranted for further clinical development of anlotinib for precise treatment of GBM in different line settings and combination regimens, such as immunotherapy.

Acknowledgements

We are thankful to the investigators, patients enrolled in this clinical trial, and patient families.

Grant support

This work was supported by the National Natural Science Foundation of China (Grant No. 82272744), Natural Science Foundation of Guangdong Province (Grant No. 2022A1515010814), and Sun Yat-sen University Clinical Research 5010 Program (Grant No. 2022008).

Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

Conceived and designed the analysis: Yuanyuan Chen, Ming Chen.
Collected the data: Fagui Jiang, Xiaohui Liu, Peijing Li, Shuzhen Lai.
Contributed data or analysis tools: Fagui Jiang, Xiaohui Liu, Guihong Liu, Jing Huang, Yiqiang Tang, Zhigang Liu, Guoping Shen, Chaoming Li, Lei Wang, Tieming Xie, Fangxiao Lu, Xing Zhang, Xiaoxuan Wang, Caixing Sun.
Performed the analysis: Shuzhen Lai, Peijing Li, Xing Zhang, Xiaoxuan Wang, Yuanyuan Chen, Ming Chen.
Wrote the paper: Shuzhen Lai, Peijing Li.

Data availability statement

Data was generated by the authors and available upon reasonable request.

References

1. Ostrom QT, Cioffi G, Gittleman H, Patil N, Waite K, Kruchko C, et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2012-2016. Neuro Oncol. 2019; 21: v1-100.
2. Zhang K, Liu X, Li G, Chang X, Li S, Chen J, et al. Clinical management and survival outcomes of patients with different molecular subtypes of diffuse gliomas in China (2011-2017): a multicenter retrospective study from CGGA. Cancer Biol Med. 2022; 19: 1460-76.
3. Xu C, Yuan X, Hou P, Li Z, Wang C, Fang C, et al. Development of glioblastoma organoids and their applications in personalized therapy. Cancer Biol Med. 2023; 20: 353-68.
4. Cui X, Wang Y, Zhou J, Wang Q, Kang C. Expert opinion on translational research for advanced glioblastoma treatment. Cancer Biol Med. 2023; 20: 344-52.
5. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med. 2005; 352: 987-96.
6. Furnari FB, Fenton T, Bachoo RM, Mukasa A, Stommel JM, Stegh A, et al. Malignant astrocytic glioma: genetics, biology, and paths to treatment. Genes Dev. 2007; 21: 2683-710.
7. Jain RK, di Tomaso E, Duda DG, Loeffler JS, Sorensen AG, Batchelor TT. Angiogenesis in brain tumours. Nat Rev Neurosci. 2007; 8: 610-22.
8. Gilbert MR, Dignam JJ, Armstrong TS, Wefel JS, Blumenthal DT, Vogelbaum MA, et al. A randomized trial of bevacizumab for newly diagnosed glioblastoma. N Engl J Med. 2014; 370: 699-708.
9. Chinot OL, Wick W, Mason W, Henriksson R, Saran F, Nishikawa R, et al. Bevacizumab plus radiotherapy-temozolomide for newly diagnosed glioblastoma. N Engl J Med. 2014; 370: 709-22.
10. Mellinghoff IK, Lassman AB, Wen PY. Signal transduction inhibitors and antiangiogenic therapies for malignant glioma. Glia. 2011; 59: 1205-12.
11. Lee EQ, Kaley TJ, Duda DG, Schiff D, Lassman AB, Wong ET, et al. A multicenter, phase II, randomized, noncomparative clinical trial of radiation and temozolomide with or without vandetanib in newly diagnosed glioblastoma patients. Clin Cancer Res. 2015; 21: 3610-8.
12. Hottinger AF, Ben Aissa A, Espeli V, Squiban D, Dunkel N, Vargas MI, et al. Phase I study of sorafenib combined with radiation therapy and temozolomide as first-line treatment of high-grade glioma. Br J Cancer. 2014; 110: 2655-1.
13. Shen G, Zheng F, Ren D, Du F, Dong Q, Wang Z, et al. Anlotinib: a novel multi-targeting tyrosine kinase inhibitor in clinical development. J Hematol Oncol. 2018; 11: 120.
14. Sun X, Zhang R, Xu J, Xie L, Guo W. First line anlotinib plus liposomal doxorubicin for locally advanced or metastatic soft tissue sarcoma: a prospective, single-arm trial. Asia Pac J Trop Med. 2022; 15: 266-73.
15. Jiang S, Liang H, Liu Z, Zhao S, Liu J, Xie Z, et al. The Impact of anlotinib on brain metastases of non-small cell lung cancer: post

hoc analysis of a phase III randomized control trial (ALTER0303). Oncologist. 2020; 25: e870-4.
16. Li PJ, Lai SZ, Jin T, Ying HJ, Chen YM, Zhang P, et al. Radiotherapy opens the blood-brain barrier and synergizes with anlotinib in treating glioblastoma. Radiother Oncol. 2023; 183: 109633.
17. Garcia J, Hurwitz HI, Sandler AB, Miles D, Coleman RL, Deurloo R, et al. Bevacizumab (Avastin®) in cancer treatment: A review of 15 years of clinical experience and future outlook. Cancer Treat Rev. 2020; 86: 102017.
18. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, et al. The 2016 World Health Organization Classification of Tumors of the Central Nervous System: a summary. Acta Neuropathol. 2016; 131: 803-20.
19. Lai Z, Markovets A, Ahdesmaki M, Chapman B, Hofmann O, McEwen R, et al. VarDict: a novel and versatile variant caller for next-generation sequencing in cancer research. Nucleic Acids Res. 2016; 44: e108.
20. Li Q, Wang K. InterVar: clinical interpretation of genetic variants by the 2015 ACMG-AMP Guidelines. Am J Hum Genet. 2017; 100: 267-80.
21. Talevich E, Shain AH, Botton T, Bastian BC. CNVkit: genome-wide copy number detection and visualization from targeted DNA sequencing. PLoS Comput Biol. 2016; 12: e1004873.
22. Newman AM, Bratman SV, Stehr H, Lee LJ, Liu CL, Diehn M, et al. FACTERA: a practical method for the discovery of genomic rearrangements at breakpoint resolution. Bioinformatics. 2014; 30: 3390-3.
23. Curran WJ Jr, Scott CB, Horton J, Nelson JS, Weinstein AS, Fischbach AJ, et al. Recursive partitioning analysis of prognostic factors in three Radiation Therapy Oncology Group malignant glioma trials. J Natl Cancer Inst. 1993; 85: 704-10.
24. Brandes AA, Franceschi E, Paccapelo A, Tallini G, De Biase D, Ghimenton C, et al. Role of MGMT methylation status at time of diagnosis and recurrence for patients with glioblastoma: clinical implications. Oncologist. 2017; 22: 432-7.
25. Berger MF, Mardis ER. The emerging clinical relevance of genomics in cancer medicine. Nat Rev Clin Oncol. 2018; 15: 353-65.
26. Sandmann T, Bourgon R, Garcia J, Li C, Cloughesy T, Chinot OL, et al. Patients with proneural glioblastoma may derive overall survival benefit from the addition of bevacizumab to first-line radiotherapy and temozolomide: retrospective analysis of the AVAglio trial. J Clin Oncol. 2015; 33: 2735-44.
27. Zhao YR, Wang JL, Xu C, Li YM, Sun B, Yang LY. HEG1 indicates poor prognosis and promotes hepatocellular carcinoma invasion, metastasis, and EMT by activating Wnt/β-catenin signaling. Clin Sci (Lond). 2019; 133: 1645-62.
28. Álvarez-Satta M, Matheu A. Primary cilium and glioblastoma. Ther Adv Med Oncol. 2018; 10: 1758835918801169.
29. Goranci-Buzhala G, Mariappan A, Ricci-Vitiani L, Josipovic N, Pacioni S, Gottardo M, et al. Cilium induction triggers differentiation of glioma stem cells. Cell Rep. 2021; 36: 109656.
30. Li D, Chi Y, Chen X, Ge M, Zhang Y, Guo Z, et al. Anlotinib in locally advanced or metastatic medullary thyroid carcinoma: a randomized, double-blind phase IIB trial. Clin Cancer Res. 2021; 27: 3567-75.
31. Sugita K, Kawakami K, Yokokawa T, Mae Y, Toya W, Hagino A, et al. Investigation of regorafenib-induced hypothyroidism in patients with metastatic colorectal cancer. Anticancer Res. 2015; 35: 4059-62.
32. Patel S, Dushenkov A, Jungsuwadee P, Krishnaswami A, Barac A. Team-based approach to management of hypertension associated with angiogenesis inhibitors. J Cardiovasc Transl Res. 2020; 13: 463-77.
33. Lai A, Tran A, Nghiemphu PL, Pope WB, Solis OE, Selch M, et al. Phase II study of bevacizumab plus temozolomide during and after radiation therapy for patients with newly diagnosed glioblastoma multiforme. J Clin Oncol. 2011; 29: 142-8.

Cite this article as: Lai S, Li P, Liu X, Liu G, Xie T, Zhang X, et al. Efficacy and safety of anlotinib combined with the STUPP regimen in patients with newly diagnosed glioblastoma: a multicenter, single arm, phase II trial. Cancer Biol Med. 2024; x: xx-xx. doi: 10.20892/j.issn.2095-3941.2023.0373