

Running title: Prognosis and vascular therapy in GBM

Postoperative clinical prognosis in 160 cases of glioblastoma and efficacy of vascular targeted therapy for recurrence

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Glioblastoma (GBM) is the most common primary malignant brain tumor. Concurrent chemoradiotherapy and adjuvant chemotherapy have become the standard treatment modalities after surgery. However, despite such aggressive treatment, the overall survival (OS) rate remains low, and the disease is highly prone to recurrence with an extremely poor prognosis after recurrence. Currently, there is no standard salvage treatment regimen. This study conducted a clinical prognostic analysis of 160 patients with GBM after surgery and evaluated the clinical efficacy of anti-angiogenic drugs (apatinib/bevacizumab) as salvage treatment after recurrence, providing a strong direction for future treatment. Among the 160 patients with GBM included in the study, univariate analysis showed that age, use of apatinib, adjuvant chemotherapy, and radiotherapy were significantly associated with OS, while gender, CD34 expression, Ki-67 expression, bevacizumab, and P53 expression were not significantly associated with OS. Multivariate analysis revealed that adjuvant chemotherapy, age, and radiotherapy were independent prognostic factors for OS in patients with GBM. The median overall survival of the entire cohort was 20.0 months, with 1-year, 3-year, and 5-year survival rates of 74.6%, 28.7%, and 12.4%, respectively. Analysis of the clinical efficacy of salvage chemotherapy in 65 patients with recurrent GBM showed that the combined anti-angiogenic drug group had a significant survival advantage compared to the chemotherapy-only group (33 months vs. 19 months). Among patients in the combined anti-angiogenic drug group, 36 patients achieved clinical control, with a disease control rate (DCR) of 73.47%, significantly higher than the 43.75% DCR in the control group. The chemotherapy combined with apatinib group had a significant survival advantage in OS ($p = 0.012$) and also benefited in DCR (66.67% vs. 43.75%); however, the chemotherapy combined with the bevacizumab group did not show a survival benefit in OS ($p = 0.078$).

Key words: glioblastoma; survival analysis; recurrent; apatinib; bevacizumab

GBM is the most common primary malignant brain tumor in adults, accounting for 15–20% of all intracranial tumors; its incidence increases with age [1], and it predominantly affects males around the age of 64 years (the male/female ratio is 1.6:1) [2]. GBM is mainly located in the cerebral hemispheres, most frequently in the frontal lobe [3]. Despite availability of a standard treatment regimen of maximal safe resection followed by concurrent chemoradiotherapy with temozolomide, the prognosis of patients with GBM remains poor, with a median overall survival (OS) of 14.6 months and a 5-years survival rate of less than 5% [4]. The limited efficacy of treatment is primarily due to the high diffusivity and invasiveness and complex genetic mutations of GBM [5], as well as the absence of a specific curative approach; salvage therapy after recurrence lacks standardized protocols and requires individual management based on each patient's specific condition.

GBM is characterized by excessive angiogenesis triggered by elevated levels of vascular endothelial growth factor (VEGF), which promotes tumor growth by facilitating formation of new blood vessels [6, 7]. However, this pronounced vascular phenotype is often accompanied by complex tumor–stromal interactions involving numerous types of immune cell, which may have negative effects on response to immunotherapy [8]. Moreover, the hypoxic microenvironment resulting from rapid tumor growth drives further formation of abundant new blood vessels [9]. In light of this, clinical strategies have been developed that involve use of multigenerational VEGF inhibitors to overcome resistance and enhance therapeutic efficacy.

Bevacizumab is a monoclonal antibody that disrupts VEGF signaling, thereby inhibiting angiogenesis and exerting antitumor effects [10]. On the basis of successful phase II trials, bevacizumab was approved by the U.S. Food and Drug Administration in 2009 for treatment of rGBM [11, 12]. Since then, it has become a second-line treatment option globally owing to its efficacy and favorable safety profile [13]. However, controversy remains regarding the survival benefit it confers. Apatinib mesylate, a novel small-molecule tyrosine kinase inhibitor targeting the VEGF pathway, has demonstrated tolerability and efficacy in patients with various cancers. Research regarding the therapeutic value of apatinib in GBM has been limited to date and requires further validation.

In the present study, we conducted a retrospective analysis of clinical data from 160 GBM patients to evaluate the impact of anti-angiogenic agents (apatinib/bevacizumab) on the prognosis of these patients, with the aim of gaining deeper insight into the efficacy of these agents in the management of rGBM.

Patients and methods

Study population. This was a retrospective, observational, non-interventional study. We retrospectively analyzed 160 patients with GBM who were hospitalized in our institution from January 2014 to January 2020. Patients could be included if they had 1) undergone maximal safe resection with pathological confirmation of GBM; 2) received standard-dose radiotherapy with concurrent temozolomide chemotherapy; and 3) had a Karnofsky performance status (KPS) score $\geq$ 70. Patients were excluded if they 1) had a history of secondary malignancies other than GBM; 2) did not complete adjuvant radiotherapy; 3) had insufficient follow-up data (defined as follow-up duration $<$ 6 months); or 4) were excluded for ethical reasons.

Recurrence was defined per RANO criteria for high-grade gliomas [14]: an increase in the sum of perpendicular diameters by $\geq$ 25%, or an increase in two-dimensional area (product of perpendicular diameters) by $\geq$ 10 mm². All magnetic resonance imaging (MRI) was performed every 2 months. All scans were independently reviewed by two blinded senior neuroradiologists; disagreements were resolved by consensus or a third reviewer.

Baseline information for the 160 patients included in this study comprised gender, age, KPS score, date of initial diagnosis, date of surgery, pathological markers (CD34 [15], Ki-67 [16], p53, etc.), radiotherapy plan, chemotherapy regimen and cycles, time and location of recurrence, whether salvage surgery was performed, date of last discharge, current status, and whether any treatment was received after discharge.

Treatment methods. After evaluation by neurosurgeons, maximal safe resection was performed via standard open craniotomy. No intraoperative adjunctive technologies (e.g., neuronavigation, image-guided surgery) were used. Postoperatively, patients were required to have a KPS score of at least 70. For radiotherapy (linear accelerator), patients were immobilized using a thermoplastic mask. Target volumes were delineated based on fused computed tomography/MRI images and included gross tumor volume, clinical target volume (expanded by 1.5 cm from the gross tumor

103 volume), and planning target volume (expanded by 0.3 cm from the clinical target volume). A total
104 dose of 60 Gy (2 Gy per fraction) was delivered to 95% of the planning target volume under
105 supervision by radiation oncologists.

106 Regarding chemotherapy, all patients received concurrent temozolomide during radiotherapy at a
107 dose of 75 mg/m²/day, followed by an adjuvant at 150 mg/m²/day (28-day cycles) after completion
108 of radiotherapy. MRI scans were performed every 2 months to assess treatment response. For
109 patients with confirmed recurrence, salvage therapy was initiated on the basis of individual
110 circumstances, including cisplatin (intravenous, 75 mg/m² every 3 weeks), lomustine (oral, 100-140
111 mg/m² every 6 weeks), apatinib (oral, 500 mg/day, reduced to 250 mg/day if the higher dosage was
112 not tolerated), or bevacizumab (intravenous, 10 mg/kg every 2 weeks). During treatment, blood cell
113 counts, renal function, blood pressure, and skin condition were regularly monitored, and adverse
114 reactions were managed symptomatically.

115 **Assessment of treatment efficacy.** During treatment, patients underwent cranial MRI scans every
116 two chemotherapy cycles. Treatment response was jointly evaluated by radiologists and radiation
117 oncologists according to the Response Assessment in Neuro-Oncology criteria [14] and categorized
118 as complete response (CR), partial response (PR), progressive disease (PD), or stable disease (SD).
119 Disease control rate (DCR) was calculated as follows:

120 $DCR = (CR + PR + SD) / (CR + PR + SD + PD) \times 100\%$.

121 DCR was assessed every 28 days (one cycle), with evaluation after 2 cycles. Progression-free
122 survival (PFS) was defined as the time from treatment initiation to disease progression, death from
123 any cause, or last follow-up (whichever occurred first). OS was defined as the time from treatment
124 initiation to death from any cause or last follow-up. Patients who died from non-glioma causes were
125 excluded.

126 **Safety analysis.** Adverse reactions, including hypertension, vomiting, diarrhea, leukopenia,
127 thrombocytopenia, hepatic/renal dysfunction, and rash, were graded from 1 to 5 according to
128 National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) v. 5.0
129 standards and relevant clinical guidelines. Specific grading and management strategies are detailed
130 in the Supplementary Table S1. We strictly followed NCI-CTCAE standards and institutional
131 protocols for monitoring adverse drug reactions in patients receiving antiangiogenic agents,
132 including detection (healthcare professionals continuously observed patients during medication),

133 recording (suspected adverse drug reactions were immediately documented with patient information,
134 drug name/batch number, dose, time, manifestations, and severity), assessment (using World Health
135 Organization causality assessment criteria or the Naranjo scale to exclude confounding factors),
136 treatment (measures were taken according to NCI-CTCAE grading), and reporting (data were
137 aggregated monthly and submitted via the national adverse drug reaction monitoring system).

138 **Immunohistochemical assessment.** Ki-67, CD34, MGMT promoter methylation, IDH, TERT,
139 1p/19q, and BRAF V600E results were obtained from postoperative pathology or molecular reports
140 issued by the Department of Pathology, Second Hospital of Hebei Medical University. This was a
141 retrospective, non-interventional study; no additional testing was performed for research purposes.
142 All tests followed standard diagnostic protocols [17].

143 **Data and statistical analysis.** Survival curves were generated using the Kaplan–Meier method, and
144 group differences were compared using the log-rank test. Variables with $P < 0.05$ in univariate
145 analysis were entered into multivariate Cox regression models, with multicollinearity assessed by
146 variance inflation factor (all values < 5). For missing CD34, p53, and Ki-67 data, available case
147 analysis was applied (only patients with recorded values were analyzed); sensitivity analyses
148 showed no significant baseline differences between patients with and without data (all $p > 0.05$).
149 Statistical analyses were performed using SPSS v. 23.0 and Prism, with a significance level of
150 $\alpha=0.05$.

151 **Ethics approval and consent to participate.** This study was conducted in accordance with
152 institutional ethical standards and the Declaration of Helsinki and was approved by the Ethics
153 Committee of the Second Hospital of Hebei Medical University (Approval No. 2026-R075). This
154 was a retrospective study, Written informed consent was obtained from all participants or their legal
155 guardians, and all patient data were de-identified prior to analysis.

156

157 **Results**

158 **Patients with GBM**

159 **Baseline characteristics of patients.** The 160 patients with GBM included in this study comprised
160 97 males (60.6%) and 63 females (39.4%). The age range was 18-76 years, with a median age of 52
161 years. The median follow-up time was 18.7 months. Thirty-four patients did not receive adjuvant
162 chemotherapy (Table 1).

163 **Survival analysis (160 patients with GBM).** mOS was 20.0 months (95% CI: 18.47–21.53). The
164 1-, 3-, and 5- year survival rates were 74.6%, 28.7%, and 12.4%, respectively (Figure 1A).

165 **Univariate analysis of OS in GBM patients.** To identify prognostic factors, we first performed
166 univariate analysis, including the following variables: age (≥ 50 years vs. < 50 years), gender (male
167 vs. female), CD34 expression (positive vs. negative), Ki-67 expression ($< 20\%$ vs. $\geq 20\%$), p53
168 expression (positive vs. negative), adjuvant chemotherapy (yes vs. no), use of apatinib (yes vs. no),
169 and use of bevacizumab (yes vs. no).

170 As shown in Table 2 and Figure 1, age ($p=0.027$; Figure 1B), use of apatinib ($p=0.014$; Figure 1E),
171 adjuvant chemotherapy ($p=0.014$; Figure 1C), and radiotherapy ($p=0.003$; Figure 1D) were
172 significantly associated with OS. By contrast, gender ($p=0.288$), CD34 expression ($p=0.206$; Figure
173 1G), Ki-67 expression ($p=0.126$; Figure 1H), use of bevacizumab ($p=0.187$; Figure 1F), and p53
174 expression ($p=0.663$) showed no significant association with OS. Although the association for
175 CD34 expression was not statistically significant ($p=0.206$), the survival curve suggested a potential
176 survival advantage for CD34-positive patients. Furthermore, the survival analysis for Ki-67
177 expression indicated that patients with Ki-67 $< 20\%$ had better survival outcomes than those with
178 Ki-67 $\geq 20\%$.

179 **Multivariate analysis.** Variables identified as statistically significant ($p < 0.05$) in the univariate
180 analysis were entered into a multivariate Cox proportional-hazards regression model to determine
181 independent prognostic factors. The multivariate analysis included age, adjuvant chemotherapy, use
182 of apatinib, and use of radiotherapy. The multivariate model was statistically significant ($p=0.006$).
183 The results indicated that adjuvant chemotherapy ($p=0.034$), age ($p=0.030$), and radiotherapy
184 ($p=0.035$) were independent prognostic factors for GBM patients. However, use of apatinib
185 ($p=0.078$) was not significantly associated with prolonged OS. Details are provided in Table 2.

186

187 **Salvage therapy for rGBM patients**

188 **Baseline information.** Among the 160 GBM cases collected, 65 patients with recurrent disease
189 who received salvage therapy were further analyzed. Inclusion criteria were: 1) completion of
190 standard postoperative radiotherapy and chemotherapy, including adjuvant temozolomide; 2)
191 recurrence confirmed by imaging and assessment by radiation oncology specialists; 3) receipt of
192 salvage chemotherapy without subsequent surgery or radiotherapy; and 4) regular MRI evaluation

193 with respect to treatment response.

194 Of the 65 patients receiving salvage chemotherapy, 26 received apatinib-based chemotherapy, and
195 28 received bevacizumab-based chemotherapy (five of whom received both apatinib and
196 bevacizumab during salvage treatment). These 65 patients were divided into two main groups
197 according to the use of anti-angiogenic agents during salvage therapy: a combination
198 anti-angiogenic therapy group ($n = 49$) and a chemotherapy-only group ($n=16$). The combination
199 anti-angiogenic therapy group was further divided into three subgroups based on the specific
200 salvage regimen: chemotherapy plus apatinib ($n=20$), chemotherapy plus bevacizumab ($n=23$), and
201 chemotherapy plus apatinib plus bevacizumab ($n=6$) (Table 3). There was no difference in the
202 baseline values between the two groups (Supplementary Table S2).

203 The median duration of apatinib treatment was 3 months (range: 1-7 months). Dose reduction due to
204 adverse reactions was required in 7.69% of patients receiving apatinib.

205 **Survival analysis (65 patients with rGBM).** Compared to chemotherapy alone, combination
206 anti-angiogenic therapy showed a significant survival advantage ($p=0.011$; Figure 2A), with a
207 median overall survival (mOS) of 33 months in the combination anti-angiogenic therapy group
208 versus 19 months in the chemotherapy-only group. Chemotherapy plus apatinib demonstrated a
209 significant survival benefit in terms of OS ($p=0.012$; Figure 2B), whereas chemotherapy plus
210 bevacizumab did not ($p=0.078$; Figure 2C).

211 **Clinical efficacy.** Of the 49 patients in the combination anti-angiogenic therapy group, one
212 achieved PR and 35 achieved SD. Thirteen patients experienced PD. Thus, disease control was
213 achieved in 36 patients, yielding a DCR of 73.47%. The DCR in the control (chemotherapy-only)
214 group was 43.75%, whereas that in the combination anti-angiogenic therapy group was significantly
215 higher ($p=0.028$; Table 3).

216 **Management of toxicities.** In the combination anti-angiogenic therapy group, 24 patients
217 experienced grade I/II adverse events, including nine cases of myelosuppression, six of rash, six of
218 mild gastrointestinal reactions (e.g., nausea and vomiting), and three of elevated blood pressure.
219 Two patients experienced grade III/IV adverse events, including one case of cerebellar hemorrhage.
220 This patient developed significant limb weakness after two treatment cycles, and follow-up MRI
221 revealed new hemorrhage within the lesion. After exclusion of other confounding factors, this was
222 diagnosed as a grade III adverse event. Apatinib was subsequently discontinued and the treatment

223 regimen was modified, with supportive care initiated. The other case involved a severe
224 gastrointestinal reaction.

225 In the chemotherapy-alone group, ten patients experienced grade I/II adverse events, including four
226 cases of myelosuppression and six of gastrointestinal reactions. One grade III/IV adverse event
227 (severe myelosuppression) was observed. All adverse events were alleviated with symptomatic
228 treatment, and no fatalities occurred. Statistical analysis showed no significant difference in adverse
229 events between the two groups ($p=0.888$).

230 Apatinib-related adverse effects included elevated blood pressure, vomiting, diarrhea, leukopenia,
231 rash, and hepatic/renal dysfunction. Among the 26 patients treated with apatinib, six developed
232 elevated blood pressure; in five of these cases, this was controlled with oral antihypertensive
233 medication, with only one patient developing malignant hypertension requiring drug
234 discontinuation. Two cases of myelosuppression were relieved with medication. Rash occurred in
235 five patients and gastrointestinal adverse reactions in three; these symptoms were alleviated by dose
236 reduction. In one patient, treatment was discontinued owing to minor intracranial hemorrhage. No
237 significant side-effects were observed in the remaining patients.

238 Severe adverse events potentially associated with bevacizumab include hypertension, arterial and
239 venous thrombosis, cerebral hemorrhage (including tumor hemorrhage), proteinuria, delayed wound
240 healing, and gastrointestinal perforation. However, among the 29 bevacizumab-treated patients in
241 our study, no arterial/venous thrombosis, proteinuria, or gastrointestinal perforation was observed.
242 The main side-effects were hypertension, leukopenia, and neutropenia, similar to those in the
243 chemotherapy-only group.

244

245 **Discussion**

246 GBM is the most common primary malignant central nervous system tumor and is characterized by
247 its highly aggressive nature. Concurrent and adjuvant temozolomide with radiotherapy became the
248 standard regimen for patients with newly diagnosed GBM in 2005. Regular and active adjuvant
249 chemotherapy post-surgery significantly prolongs overall patient survival. However, even with strict
250 adherence to this protocol, recurrence is almost inevitable, resulting in a median PFS of
251 approximately 6.9 months. Currently, there is no standard salvage treatment for patients with rGBM.
252 In addition to repeat surgery or re-irradiation, the primary systemic therapeutic options are

chemotherapy, targeted therapy, and immunotherapy. However, existing regimens, whether used alone or in combination, have not demonstrated a substantial improvement in patient survival rates. Notably, cytarabine combined with immune checkpoint blockade has shown anti-tumor activity in preclinical glioma models, providing a rationale for exploring similar strategies in rGBM [18]. In recent years, multiple preclinical studies and clinical trials have suggested that immunotherapy and targeted therapy could extend patient survival, with antiangiogenic agents (which inhibit the VEGF signaling pathway), in particular, showing potential efficacy. Examples include bevacizumab and ramucirumab, which are widely used in various cancers, as well as tyrosine kinase inhibitors such as sunitinib, sorafenib, and apatinib [19]. The updated Chinese clinical practice guidelines for adult diffuse gliomas emphasize molecular diagnostics and targeted therapy [20].

The factors influencing the prognosis and survival of GBM patients are complex. In this study, univariate and multivariate analyses were performed with variables including age, sex, chemotherapy, radiotherapy, CD34 expression, Ki-67 expression, use of bevacizumab, and use of apatinib. Age and chemotherapy emerged as independent risk factors affecting patient prognosis. Apatinib did not show independent prognostic significance in the multivariate model. This finding could be attributable to interactions between apatinib and other concurrent therapies, or it may have been due to the limited sample size in the subgroup analyses.

Age is a significant prognostic factor in GBM, which is associated with the biological mechanisms of cellular senescence. In the present study, the median survival of patients under 50 years old was 23 months, compared to 20 months for patients more than 50 years old. Elderly patients (≥ 70 years) are often excluded from standard clinical trials and receive less intensive treatment, and their optimal therapeutic regimen thus remains undefined. Differences in survival rates among elderly patients may also be influenced by factors such as KPS score, tumor biology, and central nervous system functional status.

Ki-67 is a commonly used biomarker that reflects proliferative activity of tumor cells, with high expression typically indicating rapid tumor progression and poor prognosis. Previous studies [21] have shown that GBM patients with low Ki-67 expression have significantly better OS (26.8 months) compared to those with high expression (15.8 months), and Ki-67 has been identified as an independent prognostic factor after gross total resection. Among the 141 patients in this study, the median survival of those with Ki-67 $\geq 20\%$ (20.0 months) was significantly lower than that of those

283 with Ki-67 < 20% (40.0 months).

284 VEGF is a potent pro-angiogenic cytokine. Under hypoxic conditions, cells activate
285 hypoxia-inducible factors to release VEGF, which in turn binds to its receptors to activate the
286 tyrosine kinase signaling pathway, thereby driving angiogenesis. In GBM, VEGF has crucial roles
287 in maintenance of tumor stem cell activity, optimization of the tumor microenvironment, and
288 promotion of neovascularization. As angiogenesis is a key mechanism for tumor blood supply and
289 growth [22], its inhibition by blockade of the VEGF signaling pathway represents an important
290 therapeutic strategy.

291 Bevacizumab is among the most extensively studied drugs with respect to treatment of rGBM. In
292 early clinical trials, bevacizumab combined with irinotecan achieved an objective response rate of
293 42% and increased the 6-month PFS rate to 46%; the corresponding response rates for radiotherapy
294 alone and salvage chemotherapy were only 9-21% and 4-9%, respectively [23, 24]. In 2009, the
295 FDA granted accelerated approval for bevacizumab in rGBM based on two phase II studies. By
296 contrast, the European Medicines Agency did not approve it, citing a lack of control groups,
297 limitations regarding response assessment criteria, and difficulties in interpreting OS and PFS data
298 in the relevant studies [25]. Large-scale clinical studies and meta-analyses in recent years have
299 indicated that although bevacizumab significantly prolongs PFS in rGBM patients, it does not
300 significantly improve OS. However, the drug has a clear benefit in terms of reducing intracranial
301 pressure, alleviating peritumoral edema, and improving patients' neurological function and quality
302 of life. Moreover, bevacizumab extended the median OS of rGBM patients to 23.7 months in a
303 retrospective analysis of 202 patients [26]. Therefore, bevacizumab is currently regarded as an
304 important palliative treatment option for rGBM.

305 Apatinib is a highly selective oral VEGFR-2 tyrosine kinase inhibitor that exerts antitumor effects
306 by blocking angiogenesis-related signaling pathways. It has been approved in China for treatment of
307 advanced gastric cancer [27] and has demonstrated survival benefits in various solid tumors,
308 including liver cancer, ovarian cancer, and nonsmall-cell lung cancer [28-30] Furthermore, studies
309 have shown that apatinib can reverse multidrug resistance and enhance the efficacy of conventional
310 chemotherapeutic agents including temozolomide [31, 32] .A clinical trial evaluating the salvage
311 therapeutic effect of apatinib combined with temozolomide in patients with rGBM reported an
312 objective response rate of 26.3%, a DCR of 84.2%, and median PFS and mOS of 4.9 months and

8.2 months, respectively, suggesting that this combination regimen provides clinical benefit [33].

Another retrospective analysis of 108 patients with recurrent malignant glioma showed that apatinib combined with temozolomide significantly improved objective response rate ($p=0.033$) and DCR ($p=0.036$) compared with temozolomide alone, as well as prolonging median PFS (11.7 months vs. 8.5 months) and mOS (7.4 months vs. 4.9 months) [34]. In addition, a meta-analysis of ten studies involving 357 patients with recurrent high-grade glioma also found that apatinib combined with temozolomide could improve treatment efficacy and prognosis [35]. Thus, current evidence suggests that apatinib demonstrates disease control capability and survival benefits in the treatment of recurrent high-grade glioma.

In the present study, anti-angiogenic agents combined with chemotherapy significantly improved patient survival compared with chemotherapy alone, with a median OS of 33 months in the combination group versus 19 months in the chemotherapy-only group. The DCR was also higher in the combination group, and the two groups had similar incidence of adverse reactions. As some patients received both apatinib and bevacizumab during salvage therapy, we further performed a stratified analysis of the combination treatment group. Chemotherapy combined with apatinib showed a more favorable trend in terms of survival benefit compared to chemotherapy combined with bevacizumab. However, owing to the high heterogeneity of the salvage treatment regimens, it was difficult to conduct a sufficient stratified comparison between the two antiangiogenic agents (apatinib vs. bevacizumab). Future large-scale prospective studies are therefore needed to clarify the efficacy of apatinib in rGBM.

This retrospective study has limitations. This retrospective study has several limitations. First, data were missing for CD34 (18.7%), p53 (27.5%), and Ki-67 (11.9%) due to non-routine clinical ordering or limited specimens. Although available case analysis was used, the high missing proportion—especially for p53—may introduce bias. Second, PFS data were missing for some patients due to variable treatment adherence. Third, molecular subgroup sample sizes were too small for robust analysis (IDH mutant: 3; MGMT methylated: 15; TERT mutant: 28; 1p/19q codeletion: 0; BRAF V600E mutant: 2), consistent with common real-world challenges [36]. Larger studies are needed to validate our findings. Nonetheless, we performed sensitivity analyses (intention-to-treat and per-protocol) and excluded crossover patients to minimize bias.

Most other anti-angiogenic agents (e.g., VEGFR-TKIs, PKC inhibitors) have shown insufficient

343 efficacy in GBM trials [37, 38] and resistance is common. Recent evidence indicates that VEGF
344 blockade alone may be insufficient for effective vascular pruning, requiring combination with other
345 targets [39]. Emerging reviews have detailed the multifaceted resistance mechanisms to
346 anti-angiogenic therapy in GBM, including activation of redundant pro-angiogenic pathways,
347 vasculogenic mimicry, and hypoxia-induced invasion [40, 41]. Furthermore, hypoxia induces
348 GSC-to-endothelial transdifferentiation via the ROR1-WNT5A axis, promoting vascularization and
349 anti-angiogenic resistance [42]. Contemporary reviews advocate for multi-targeted and combination
350 strategies to overcome these adaptive resistance mechanisms [41, 43]. Therefore, elucidating
351 resistance mechanisms and developing novel agents targeting alternative pathways remain key
352 priorities for improving long-term efficacy [44].

353 In conclusion, our univariate analysis showed that age, adjuvant chemotherapy, and use of apatinib
354 were associated with prognosis of GBM patients, with CD34 and Ki-67 expression levels also
355 demonstrating reference value. Multivariate analysis further confirmed that age and chemotherapy
356 were independent prognostic factors for GBM patients. In patients with rGBM, we found that
357 apatinib could prolong OS and improve short-term efficacy with good tolerability. Currently, there
358 is no standard treatment regimen for rGBM, and patient prognosis remains poor. Therefore, active
359 exploration of effective treatment strategies to prolong survival and improve quality of life is of
360 great importance. Finally, well-designed prospective studies with larger cohorts and standardized
361 molecular profiling are warranted to confirm our findings, as well as to further elucidate resistance
362 mechanisms and develop rational combination therapies with other targeted agents.

363

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368 **Supplementary data are available in the online version of the paper.**

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Figure Legends

Figure 1. Kaplan-Meier survival analysis of overall survival (OS). Kaplan-Meier curves illustrate the associations between clinicopathological factors and OS. Age (B), adjuvant chemotherapy (C), radiotherapy (D), and use of apatinib (E) were significantly associated with OS. In contrast, gender (A), use of bevacizumab (F), CD34 expression (G), Ki-67 expression (H), and p53 expression (I) showed no statistically significant associations with OS.

Figure 2. Kaplan-Meier curves of overall survival (OS) according to anti-angiogenic treatment strategies. Kaplan-Meier survival curves comparing OS between chemotherapy alone and combination anti-angiogenic therapy (A), chemotherapy plus apatinib (B), and chemotherapy plus bevacizumab (C).

531 **Table 1.** Clinical information of 160 patients with glioblastoma.

clinical features	Number (%)
Age, yr	
< 50	70 (43.7%)
≥ 50	90 (56.3%)
Gender, n (%)	
Male	97 (60.6%)
Female	63 (39.4%)
Adjuvant chemotherapy	
Yes	126 (78.7%)
No	34 (21.3%)
CD34	
Positive	76 (47.5%)
Negative	54 (33.8%)
NA	30 (18.7%)
P53	
Positive	81 (50.6%)
Negative	35 (21.9%)
NA	44 (27.5%)
Ki67	
< 20%	18 (11.2%)
≥ 20%	123 (76.9%)
NA	19 (11.9%)
apatinib use	
Yes	30 (18.8%)
No	130 (81.2%)
BEV use	
Yes	36 (22.5%)
No	124 (77.5%)

532 Abbreviations: NA-not available

533

534 **Table 2.** Results of univariate and multivariate analysis of glioblastoma patients and overall
535 survival.

Factor	Median Survival time	Univariate analysis		Multivariate analysis	
		HR (95%CI)	p-value	HR (95%CI)	p-value
Age , yr			0.027	1.630 (1.049-2.532)	0.030
< 50	23.00 m	0.629 (0.414-0.955)			
≥ 50	20.00 m	1.591 (1.047-2.416)			
Gender n (%)			0.288		
Male	20.00 m	1.258 (0.824-1.922)			
Female	21.00 m	0.795 (0.520-1.214)			
Adjuvant chemotherapy			0.014	1.808 (1.046-3.113)	0.034
Yes	21.00 m	0.535 (0.282-1.016)			
No	11.00 m	1.868 (0.985-3.546)			
Radiotherapy			0.003	2.362 (1.061-5.257)	0.035
Yes	21.00 m	0.332 (0.094-1.181)			
No	8.00 m	3.010 (0.847-10.70)			
CD34			0.206		
Positive	22.00 m	0.746 (0.463-1.201)			
Negative	21.00 m	1.341 (0.833-2.159)			
P53			0.663		
Positive	21.00 m	0.880 (0.477-1.622)			
Negative	20.00 m	1.137 (0.617-2.157)			
Ki67			0.126		
< 20%	40.00 m	0.560 (0.299-1.045)			
≥ 20%	20.00 m	1.787 (0.957-3.338)			
apatinib use			0.014	1.688 (0.943-3.022)	0.078
Yes	33.00 m	0.518 (0.326-0.822)			
No	20.00 m	1.932 (1.216-3.071)			

BEV use			0.187
Yes	22.00 m	0.713 (0.444-1.144)	
No	20.00 m	1.403 (0.874-2.253)	

536 Abbreviations: m-months

537

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538 **Table 3.** Clinical efficacy results.

Group	Number	CR	PR	SD	PD	DCR
Chemotherapy+anti-angiogenic therapy group	49	0	1	35	13	73.47%
Chemotherapy+apatinib group	21	0	0	14	7	66.67%
Chemotherapy+BEV group	23	0	0	18	5	78.26%
Chemotherapy+BEVsequential Apatinib group	5	0	1	3	1	80.00%
Chemotherapy alone group	16	0	1	6	9	43.75%
X²						4.758
p-value						0.029

539

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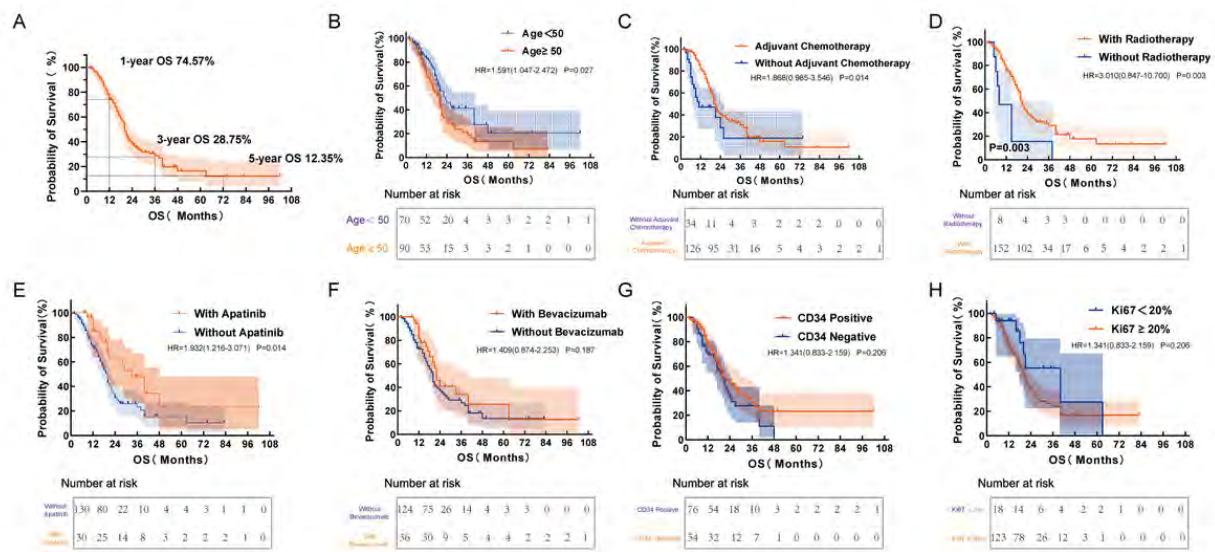


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