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Original Article

Efficacy and survival outcomes of bevacizumab plus minocycline for glioblastoma

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Abstract: Objective: To evaluate the efficacy and survival outcomes of bevacizumab combined with minocycline versus bevacizumab monotherapy in patients with glioblastoma (GBM). Methods: We conducted a retrospective analysis of 132 GBM patients treated at multiple centers between January 2022 and December 2023. Patients were divided into a control group (bevacizumab monotherapy, n = 67) and an observation group (bevacizumab plus minocycline, n = 65). Short-term treatment response, serum biomarkers, immune function, inflammatory and angiogenic factors, quality of life, safety, and long-term survival were assessed. Results: The observation group showed significantly higher objective response rate (53.85% vs. 29.85%) and disease control rate (78.46% vs. 61.19%), along with improved immune function, reduced inflammatory and angiogenic markers, and enhanced quality of life (all $P < 0.05$). Median progression-free survival (PFS) (8.5 vs. 6.7 months) and overall survival (OS) (10.6 vs. 8.9 months) were longer in the observation group. No significant difference in treatment-related adverse events was observed. Conclusion: This retrospective analysis suggests that the combination of bevacizumab and minocycline is associated with promising efficacy in GBM patients, including improved objective response, survival, and quality of life, with a manageable safety profile. These findings support further evaluation in prospective randomized trials to confirm the therapeutic potential of this combination.

Keywords: Glioblastoma, bevacizumab, minocycline, survival outcomes, combination therapy

Introduction

Glioblastoma (GBM), the most common and aggressive primary brain tumor in adults, is characterized by rapid proliferation, significant heterogeneity, and high invasiveness [1]. Although current multimodal treatments - including surgical resection, radiotherapy, and chemotherapy - can delay tumor progression to some extent, they have failed to substantially improve the high recurrence rate and poor survival outcomes associated with GBM. Thus, the prognosis for patients remains dismal [2]. Thus, there is an urgent need for novel therapeutic strategies to improve outcomes.

In response to this urgent clinical need, research has increasingly focused on immunotherapy and targeted therapy. This has promoted innovation in treatment approaches for GBM [3]. Among these, bevacizumab, a monoclonal antibody targeting angiogenesis, has been shown to inhibit tumor vascularization and modulate the tumor microenvironment. It can thereby delay tumor growth and recurrence [4]. By binding to vascular endothelial growth factor (VEGF), bevacizumab blocks its interaction with VEGF receptors. This reduces the formation of new blood vessels and has demonstrated clinical benefits, particularly in the second-line treatment of GBM [5]. Meanwhile, minocycline, a broad-spectrum antibiotic with excellent central nervous system penetration, has gained increasing attention for its potential use in brain tumor therapy [6]. Beyond its antibacterial effects, minocycline exhibits multiple antitumor mechanisms. These include immunomodulation, induction of apoptosis, and su-

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ppression of tumor-associated inflammation [7]. Furthermore, its ability to cross the blood-brain barrier is superior to other tetracycline analogs. This makes it a promising candidate for treating brain malignancies [8]. Although the combined use of bevacizumab and minocycline has not been fully evaluated in clinical settings, their potential synergy may improve treatment efficacy for GBM. This combination could achieve dual inhibition of angiogenesis, modulate the tumor immune microenvironment, and suppress tumor invasion. It may offer a new therapeutic alternative for patients.

This study was designed to investigate the efficacy and survival outcomes of bevacizumab combined with minocycline in GBM, and to evaluate the safety and effectiveness of this combination regimen, with the aim of providing a rationale for its broader clinical application.

Materials and methods

Study population

We retrospectively enrolled 132 patients with GBM treated at Shandong Provincial Third Hospital, Affiliated Tumor Hospital of Shandong First Medical University, Affiliated Central Hospital of Shandong First Medical University, and Fuding Hospital Affiliated to Fujian University of Traditional Chinese Medicine between January 2022 and December 2023. Patients were divided into a control group (bevacizumab monotherapy, n = 67) and an observation group (bevacizumab combined with minocycline, n = 65). Inclusion criteria were: (1) age 18-65 years; (2) no contraindications to bevacizumab or minocycline; (3) clinical stage II/III disease. Exclusion criteria included: (1) other malignancies; (2) expected survival < 3 months; (3) severe psychiatric or cognitive impairment; (4) significant organ dysfunction. The study protocol received approval from the Institutional Ethics Committee of Shandong Provincial Third Hospital (Approval No.: KYLL-2025200).

Sample size

Prior to the study, a sample size calculation was performed using the log-rank test for survival data. The assumed hazard ratio (HR) of 0.60 for progression-free survival (PFS) in the combination group was based on effect sizes reported in pivotal trials of combination therapies involving bevacizumab in other solid tumors, where HRs around 0.60-0.65 were observed for survival endpoints [9]. With a two-sided significance level (α) of 0.05 and a statistical power (1-β) of 80%, the calculation indicated that a total of approximately 120 patients (60 per group) would be required. Accounting for an estimated 10% dropout rate, we aimed to enroll a total of 132 patients. Thus, the final sample size of 132 patients is considered adequate to detect the anticipated clinically meaningful treatment effect.

Treatment protocols

Patients in the observation and control groups received conventional radiotherapy, which was initiated 2-4 weeks following surgery. Immobilization was achieved with thermoplastic masks, and treatment planning was performed via CT simulation. All patients received intensity-modulated radiation therapy (IMRT). The clinical target volume (CTV) was defined based on preoperative MR images and any postoperative residual lesions, and included the gross tumor volume (GTV) and/or the surgical cavity with a 2-3 cm margin, incorporating the surrounding edema region visible on MRI. The planning target volume (PTV) was created by adding a 0.5 cm margin to the CTV, with the constraint that ≥ 95% of the PTV received the prescription dose. The initial dose regimen was 1.8 Gy per fraction, delivered 5 times per week. After a total dose of 50.4 Gy in 28 fractions, the radiation field was reduced to cover the GTV plus a 0.5-1.0 cm margin. An additional boost dose of 10 Gy in 5 fractions was then administered at 2.0 Gy per fraction, 5 times per week, bringing the total dose to 60.4 Gy in 33 fractions. This regimen, which delivers a comparable total dose using smaller fraction sizes, represents a clinical adaptation to optimize the therapeutic ratio. This approach aligns with contemporary guidelines that acknowledge the use of hypofractionated and adapted schedules in GBM radiotherapy, based on individual clinical considerations and institutional protocols [10].

In the control group, bevacizumab was administered intravenously at 5 mg/kg on day 1 of each cycle. The drug was diluted in 250 mL of normal saline and infused over 30 to 90 minutes, based on patient tolerance. Treatment cycles were repeated every two weeks for a total of 6 cycles (3 months). The observation group additionally received oral minocycline hydrochloride capsules at 100 mg twice daily

for 3 months. This dosing regimen was informed by prior preclinical studies in glioma models, where minocycline demonstrated antitumor efficacy and provided a rationale for its clinical investigation [11], and is supported by its established clinical safety and central nervous system penetration profile [6, 8].

Baseline data and clinical evaluation

Upon hospital admission, baseline data including gender, age, body mass index (BMI), clinical stage, place of residence, marital status, average monthly household income, and educational level were collected from the patients. Short-term treatment efficacy was evaluated after the completion of chemotherapy, according to the criteria established in the *Chinese expert consensus on immunotherapy and targeted therapy for gliomas in the central nervous system (2nd edition)* [12]. The criteria were defined as follows: complete response (CR): disappearance of all target lesions; partial response (PR): ≥ 30% reduction in tumor diameter; stable disease (SD): reduction in tumor volume that did not meet the criteria for PR, or increase that did not meet the criteria for progressive disease; progressive disease (PD): ≥ 20% increase in tumor diameter or emergence of new lesion(s). The objective response rate (ORR) was calculated as (CR + PR)%, and the disease control rate (DCR) as (CR + PR + SD)%.

Serum biomarker assays

To minimize pre-analytical variations, all venous blood samples were collected under standardized conditions. The sampling time was uniformly set as early in the morning (7:00-9:00) with an overnight fasting state. Samples before treatment were obtained before starting any study drug treatment, while samples during the treatment period were collected at fixed time points according to the bevacizumab infusion cycle (immediately before the next scheduled administration). Samples were centrifuged within 2 hours after collection (at 2°C, 3,000 rpm for 10 minutes with a rotor radius of 8 cm) to separate serum, which was then aliquoted into three portions. One aliquot was used to determine the levels of matrix metalloproteinase-2 (MMP-2), matrix metalloproteinase-8 (MMP-8), matrix metalloproteinase-13 (MMP-13), and tissue inhibitor of metalloproteinase-1 (TIMP-1) using enzyme-linked immunosorbent assay (ELISA) kits purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd. Another aliquot was analyzed using a DxP Athena flow cytometer (Qingdao Jiading Analytical Instrument Co., Ltd.) to assess immune function markers, including the levels of Cluster of Differentiation 3 positive ($CD3^+$), Cluster of Differentiation 4 positive ($CD4^+$), and Cluster of Differentiation 8 positive ($CD8^+$) T lymphocytes. The $CD4^+/CD8^+$ ratio was then calculated. All kits were procured from Beijing Everbridge Medical Co., Ltd. The third aliquot was subjected to ELISA to quantify the levels of inflammatory cytokines [interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), and leukotriene B4 (LTB4)] as well as angiogenesis factors [vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and transforming growth factor-β1 (TGF-β1)]. Measurements were performed using an AEW-96S multifunctional microplate reader (Shanghai Shiwei Laboratory Instrument Technology Co., Ltd.).

Quality of life, safety, and long-term efficacy assessment

Quality of life was assessed using the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire-Core 30 (EORTC QLQ-C30) [13] on the day before chemotherapy initiation and one day after chemotherapy completion. The physical functioning (PF), role functioning (RF), cognitive functioning (CF), and emotional functioning (EF) domains were evaluated, each scored on a scale of 0 to 100, with higher scores indicating better quality of life. Treatment safety was monitored by recording treatment-related adverse events occurring during treatment, including vomiting/diarrhea, bone marrow suppression, anemia, abnormal liver function, rash, dizziness, skin pigmentation, headache/visual changes, and other potential complications. Long-term efficacy was evaluated through follow-up visits conducted monthly via outpatient clinic or telephone for one year after the initiation of treatment. PFS was defined as the time from treatment initiation to disease progression, and overall survival (OS) was defined as the time from treatment initiation to death from any cause.

Statistical analysis

Data were analyzed using SPSS 25.0 and GraphPad Prism 9.5. Normally distributed con-

Table 1. Comparison of baseline characteristics between control and observation groups

Characteristic	Control (n = 67)	Observation (n = 65)	t/χ^2	*P*
Gender (n, %)			0.141	0.707
Male	37 (55.22)	38 (58.46)		
Female	30 (44.78)	27 (41.54)		
Age (years, $\bar{x} \pm s$)	40.66 ± 7.44	41.35 ± 8.00	0.519	0.605
BMI (kg/m^2, $\bar{x} \pm s$)	22.69 ± 2.82	22.68 ± 3.02	-0.024	0.981
Clinical Staging (n, %)			0.339	0.844
IIa	26 (38.81)	28 (43.08)		
IIb	23 (34.33)	22 (33.85)		
III	18 (26.86)	15 (23.07)		
Residence (n, %)			0.152	0.696
Urban	39 (58.21)	40 (61.54)		
Rural	28 (41.79)	25 (38.46)		
Marital Status (n, %)			1.481	0.477
Never married	2 (2.99)	5 (7.69)		
Married	58 (86.56)	53 (81.54)		
Divorced/Widowed	7 (10.45)	7 (10.77)		
Monthly household income (n, %)			0.255	0.614
≤ 5,000	28 (41.79)	30 (46.15)		
> 5,000	39 (58.21)	35 (53.85)		
Education level (n, %)			0.588	0.443
High school or below	40 (59.70)	43 (66.15)		
College or above	27 (40.30)	22 (33.85)		

Note: BMI: body mass index.

tinuous data were expressed as mean ± standard deviation and analyzed using paired t-tests for within-group comparisons and independent t-tests for between-group comparisons. Non-normally distributed data were presented as median and interquartile range, with within-group comparisons conducted using the Wilcoxon signed-rank test and between-group comparisons analyzed using the Kruskal-Wallis H test. Categorical data were summarized as frequency and percentage (n, %), and group differences were assessed using the chi-square test. Survival analysis was performed using the Kaplan-Meier method, and survival curves were compared with the log-rank test. A *P*-value < 0.05 was considered statistically significant.

Results

Comparison of baseline characteristics

No significant differences were observed between the two groups in gender, age, BMI, clinical stage, residence, marital status, income, or education level (all $P > 0.05$), as detailed in **Table 1**.

Comparison of short-term treatment response between the two groups

The proportion of patients achieving CR and PR was higher in the observation group compared to the control group ($P < 0.05$; **Table 2**). Furthermore, both the ORR and DCR were significantly greater in the observation group, with all differences being statistically significant ($P < 0.05$; **Table 2**).

Comparison of tumor invasion markers between the two groups

Baseline levels of MMP-2, MMP-8, MMP-13, and TIMP-1 were comparable between the two groups (all $P > 0.05$; **Figure 1**). Following treatment, significant improvements in all markers were observed within each group relative to their baseline levels. Compared with the control group, the observation group exhibited a

Table 2. Comparison of short-term treatment response between the control and observation groups (n, %)

Group	n	CR	PR	SD	PD	ORR	DCR
Control	67	8 (11.94)	12 (17.91)	21 (31.34)	26 (38.81)	20 (29.85)	41 (61.19)
Observation	65	11 (16.92)	24 (36.92)	16 (24.62)	14 (21.54)	35 (53.85)	51 (78.46)
χ^2			8.721			7.816	4.658
P			0.033			0.005	0.031

Note: CR: complete response, PR: partial response, SD: stable disease, PD: progressive disease, ORR: objective response rate, DCR: disease control rate.

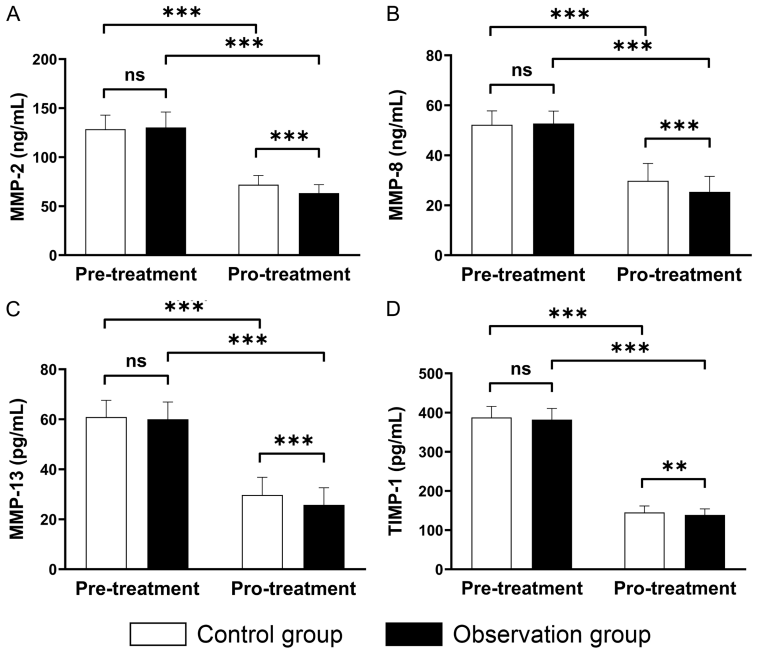

Figure 1. Comparison of tumor invasion markers between the control and observation groups. (A) MMP-2, (B) MMP-8, (C) MMP-13, (D) TIMP-1. Data are presented as mean ± standard deviation (SD). Between-group comparisons were analyzed by independent samples t-test. MMP-2 = matrix metalloproteinase-2; MMP-8 = matrix metalloproteinase-8; MMP-13 = matrix metalloproteinase-13; TIMP-1 = Tissue inhibitor of metalloproteinase-1; ns = not significant; $^{**}P < 0.01$; $^{***}P < 0.001$.

significant reduction in the concentrations of MMP-2, MMP-8, and MMP-13, and a significantly higher level of TIMP-1 (all $P < 0.05$; **Figure 1**).

Comparison of immune function indicators between the two groups

Immune function parameters, including $CD3^+$, $CD4^+$, $CD8^+$ T cell levels, and the $CD4^+/CD8^+$ ratio, were similar between groups at baseline (all $P > 0.05$; **Figure 2**). Following treatment, significant improvements in all immune parameters were observed within each group relative to their baseline levels. Compared with the control group, the observation group exhibited significantly higher levels of $CD3^+$, $CD4^+$, and $CD4^+/CD8^+$ ratio, along with a significantly lower level of $CD8^+$ (all $P < 0.05$; **Figure 2**).

Comparison of inflammatory factors between the two groups

Baseline serum levels of IL-8, TNF-α, and LTB4 were comparable between the two groups (all $P > 0.05$; **Figure 3**). Following treatment, significant reductions in the levels of these inflammatory factors were observed within each group relative to their baseline levels. Compared with the control group, the observation group exhibited significantly lower concentrations of IL-8, TNF-α, and LTB4 (all $P < 0.05$; **Figure 3**).

Comparison of angiogenic factors between the two groups

Baseline levels of VEGF, bFGF, and TGF-β1 were comparable between the two groups (all $P > 0.05$; **Figure 4**). Following treatment, a significant reduction in the levels of these angiogenic factors was observed within each group relative to baseline levels. Compared with the control group, the observation group exhibited significantly lower expression levels of VEGF, bFGF, and TGF-β1 (all $P < 0.05$; **Figure 4**).

Comparison of quality of life scores between the two groups

Quality of life scores (PF, RF, CF, and EF) were comparable between the two groups at base-

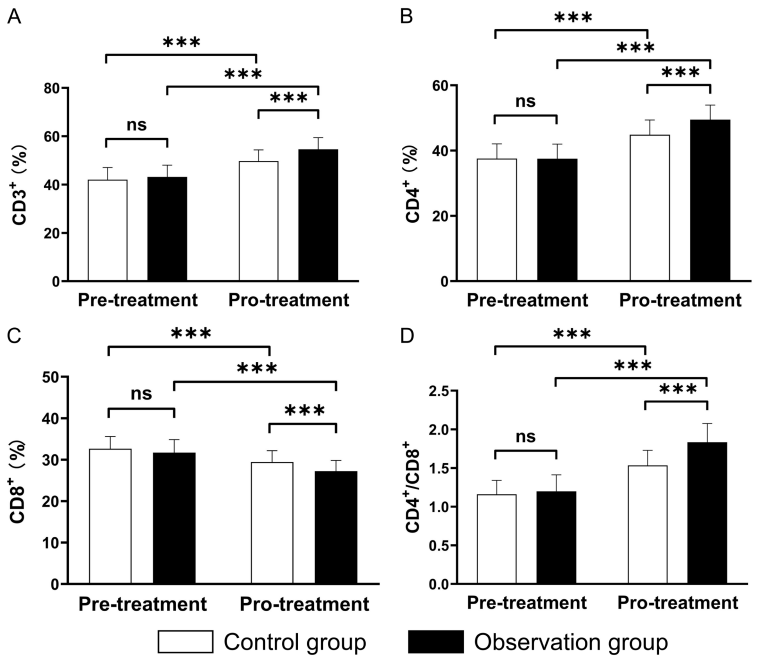

Figure 2. Comparison of immune function indicators between the control and observation groups. (A) $CD3^+$, (B) $CD4^+$, (C) $CD8^+$, (D) $CD4^+/CD8^+$. Data are presented as mean ± standard deviation (SD). Between-group comparisons were analyzed by independent samples t-test. $CD3^+$ = Cluster of differentiation 3 positive; $CD4^+$ = Cluster of differentiation 4 positive; $CD8^+$ = Cluster of differentiation 8 positive; $CD4^+/CD8^+$ = CD4 positive to CD8 positive ratio; ns = not significant; $^{***}P < 0.001$.

line (all $P > 0.05$; **Figure 5**). Following treatment, significant increases in these scores were observed within each group compared to their baseline levels. Compared with the control group, the observation group exhibited significantly greater improvements in all four dimensions (PF, RF, CF, and EF) (all $P < 0.05$; **Figure 5**).

Comparison of treatment safety between the two groups

The overall incidence of treatment-related adverse events did not differ significantly between the control and observation groups [32.84% (22/67) vs. 26.15% (17/65), respectively; $\chi^2 = 0.708$, $P = 0.400$]. In response to the specific safety concerns regarding minocycline, we performed a detailed analysis of its characteristic toxicities. As summarized in **Figure 6**, the incidences of skin pigmentation (3.08% vs. 4.48%), headache (4.62% vs. 5.97%), and hepatotoxicity (1.54% vs. 2.99%) in the observation group were all comparable to, and not significantly higher than, those in the control group (all $P > 0.05$). No confirmed cases of minocycline-induced intracranial hypertension or autoimmune hepatitis were diagnosed during the study period.

Comparison of long-term efficacy between the two groups

The median PFS was 8.5 months in the observation group and 6.7 months in the control group, while the median OS was 10.6 months and 8.9 months, respectively. Both median PFS and OS were significantly longer in the observation group compared to the control group, with a statistically significant difference ($P < 0.05$; **Figure 7**).

Discussion

GBM is the most common and aggressive primary brain tumor in adults. Due to its high recurrence rate, poor prognosis and limited treatment options, it poses significant challenges to clinical practice [14]. This multicenter retrospective study evaluated the efficacy and safety of oral minocycline combined with bevacizumab in patients with GBM. Compared with bevacizumab monotherapy, the combination therapy led to superior outcomes in ORR, DCR, PFS and OS, and demonstrated a synergistic effect in multiple biological processes including tumor invasion, immune regulation, suppression of inflammation and angiogenesis.

In this study, treatment led to a marked reduction in MMP-2, MMP-8, and MMP-13 levels and a concurrent increase in TIMP-1 in both groups ($P < 0.05$), mirroring findings from earlier studies [15]. Furthermore, significantly more patients in the observation group achieved CR, PR, ORR, and DCR compared to the control group ($P < 0.05$), indicating a potent synergistic antitumor effect between oral minocycline and bevacizumab. The observed synergy likely arises from the distinct yet complementary mechanisms of action of the two agents. The primary mechanism of bevacizumab involves inhibiting

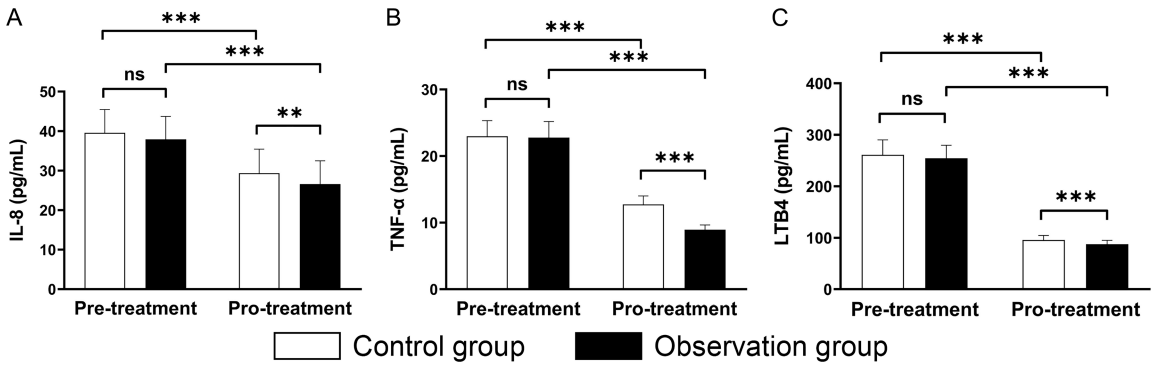

Figure 3. Comparison of inflammatory factors between the control and observation groups. Data are presented as mean ± standard deviation (SD). Between-group comparisons were analyzed by independent samples t-test. IL-8 = interleukin-8; TNF-α = Tumor necrosis factor-alpha; LTB4 = leukotriene B4; ns = not significant; $^{**}P < 0.01$; $^{***}P < 0.001$.

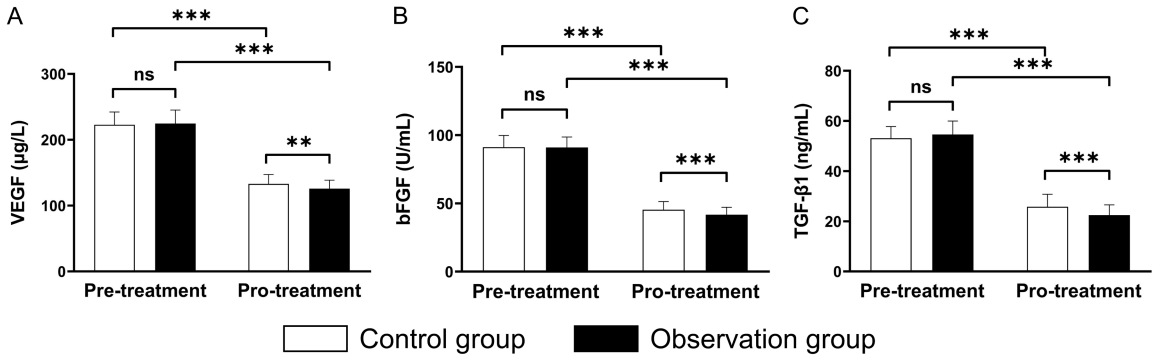

Figure 4. Comparison of angiogenic factors between the control and observation groups. (A) VEGF, (B) bFGF, (C) TGF-β1. Data are presented as mean ± standard deviation (SD). Between-group comparisons were analyzed by independent samples t-test. VEGF = vascular endothelial growth factor; bFGF = basic fibroblast growth factor; TGF-β1 = Transforming growth factor-beta 1; ns = not significant; $^{**}P < 0.01$; $^{***}P < 0.001$.

the binding of VEGF to its receptors, thereby suppressing tumor angiogenesis and growth [16]. Bevacizumab has shown synergistic effects in various cancer treatments, such as by enhancing antitumor efficacy when combined with chemotherapeutic agents like oxaliplatin [17]. Additionally, bevacizumab exerts antitumor effects by inhibiting microvessel density (MVD) and inducing tumor cell apoptosis [9, 18]. Minocycline, a tetracycline antibiotic, possesses anti-inflammatory, antimicrobial, and neuroprotective properties, and has shown inhibitory effects on tumor cells in some studies, for instance, by suppressing autophagy or inducing apoptosis [19, 20].

Matrix metalloproteinases (MMPs) play a crucial role in extracellular matrix degradation, cell migration, and tissue remodeling, and are involved in various physiological and pathological processes such as embryonic development, tissue repair, inflammation, and tumor formation [21]. Their activity is regulated by TIMPs, and the balance between MMPs and TIMPs maintains extracellular matrix homeostasis. After treatment, the levels of MMP-2, MMP-8, and MMP-13 in both groups of patients significantly decreased, while the level of TIMP-1 increased ($P < 0.05$), which is consistent with the results of earlier studies [22-24]. These changes were particularly significant in the observation group ($P < 0.05$). The decrease in MMP levels often indicates a reduction in inflammation or tissue damage, while the increase in TIMP-1 may reflect enhanced tissue repair or improved inflammation control [25]. From a mechanistic perspective, minocycline and bevacizumab jointly affect the MMP-TIMP

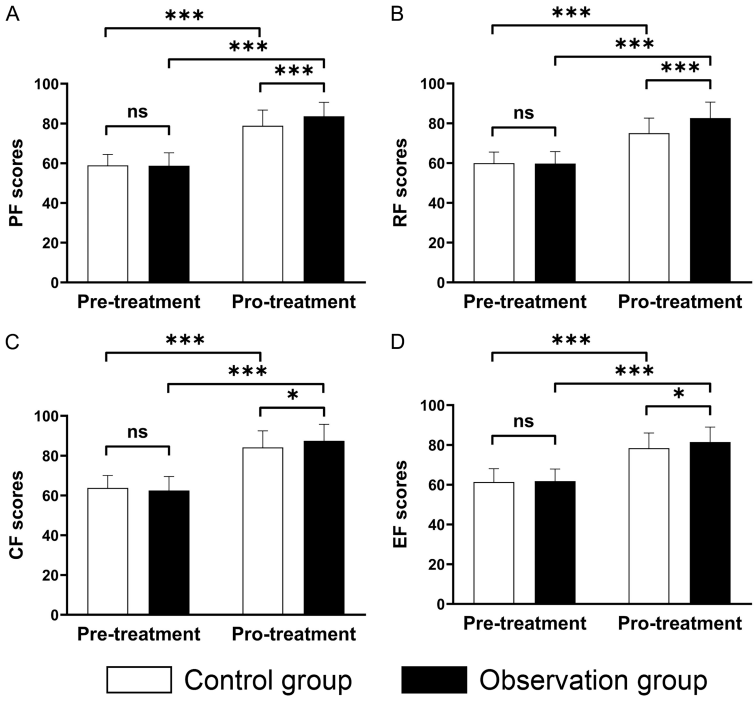

Figure 5. Comparison of quality of life scores between the control and observation groups. (A) PF, (B) RF, (C) CF, (D) EF. Data are presented as mean ± standard deviation (SD). Between-group comparisons were analyzed by independent samples t-test. PF = physical functioning; RF = role functioning; CF = cognitive functioning; EF = emotional functioning; ns = not significant; $^{*}P < 0.05$; $^{***}P < 0.001$.

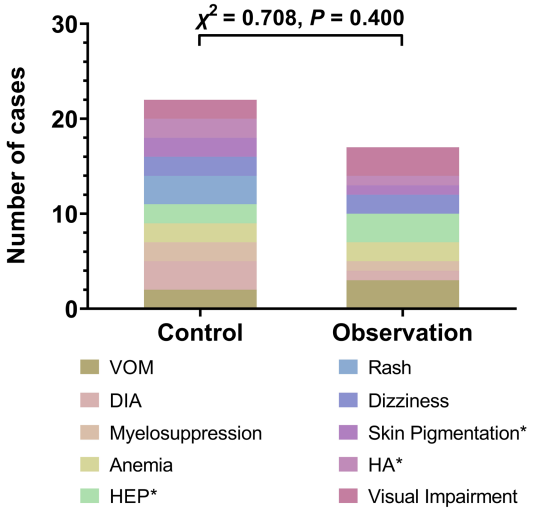

Figure 6. Comparison of treatment-related adverse events between the control and observation groups. Adverse events of special interest for minocycline (hepatotoxicity, headache, and skin pigmentation) are indicated by an asterisk (*). Between-group comparisons were analyzed by Chi-square test. VOM = Vomiting; DIA = Diarrhea; HEP = Hepatotoxicity; HA = Headache.

system through a synergistic effect: minocycline inhibits MMP activity by chelating zinc ions and downregulates the expression of MMP-2, -9, and -13 through the inhibition of the NF-κB pathway [26, 27]; bevacizumab inhibits abnormal angiogenesis and promotes vascular normalization by neutralizing VEGF, thereby improving the hypoxic state and reducing MMP expression induced by HIF-1α. Its anti-inflammatory effect can also limit myeloid cell infiltration and reduce the source of MMP [28-30]. Together, they form a multi-level inhibitory mechanism that collaboratively lowers MMP levels and elevates TIMP-1, thereby enhancing the inhibitory ability against tumor invasion.

Tumor-infiltrating lymphocytes (TILs) are mainly composed of $CD3^+$, $CD4^+$ and $CD8^+$ T cells and are the core components of anti-tumor immunity [28]. The density, distribution and $CD4^+/CD8^+$ ratio of these cells are key parameters for evaluating the tumor immune microenvironment (TIME). After treatment, we observed a significant increase in the infiltration of $CD3^+$ and $CD4^+$ T cells, an increase in the $CD4^+/CD8^+$ ratio, and a decrease in the number of $CD8^+$ T cells ($P < 0.05$). Compared with the control group, these changes were more obvious in the observation group ($P < 0.05$), which was consistent with the reported immune regulatory treatment response [29]. The decrease in $CD8^+$ T cells may reflect the post-antigenic clonal contraction or differentiation process, rather than immunosuppression [30, 31]. This dynamic pattern is consistent with the good response in other immune regulatory treatments. The significant increase in the $CD4^+/CD8^+$ ratio in the observation group suggests that bevacizumab and minocycline may work synergistically to transform the tumor immune microenvironment from an immunosuppressive state to an immune-active. The continuous increase in this ratio has been prov-

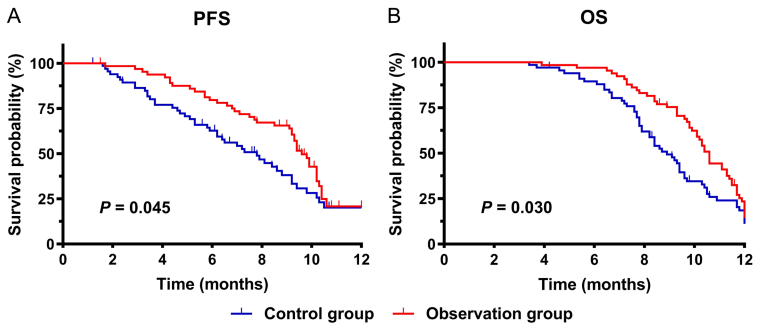

Figure 7. Comparison of long-term efficacy between the two groups. (A) PFS, (B) OS. Survival curves were compared using the log-rank test. Vertical ticks indicate censored data. PFS = progression-free survival; OS = overall survival; vertical ticks indicate censored data.

en to be a predictive indicator for a good clinical outcome [32]. The results of this study not only support the value of this ratio in evaluating the efficacy of immunotherapy for glioblastoma, but also indicate that the combined treatment strategy may improve clinical benefits by optimizing immune indicators.

Inflammatory cytokines including IL-8, TNF-α, and LTB4 are key mediators involved in angiogenesis, cell proliferation, and immune evasion within the tumor microenvironment, and play significant roles in rheumatoid arthritis, inflammatory bowel disease, and malignancies [33-35]. We observed a significant decrease in serum levels of IL-8, TNF-α, and LTB4, a finding that aligns with previous reports [36-38] and extends beyond them to underscore the superior anti-inflammatory efficacy of the combination therapy. Decreases in these cytokines typically reflect suppressed neutrophil activation, attenuated macrophage responses, and regulated leukotriene pathways, indicating effective control of inflammation [36-38]. The combination therapy acts through multiple mechanistic pathways. Minocycline suppresses NF-κB and p38 MAPK signaling, which in turn reduces the transcription and secretion of TNF-α and IL-8. It also curbs neutrophil migration and LTB4 synthesis [39, 40]. Bevacizumab neutralizes VEGF, inhibiting abnormal angiogenesis and reducing vascular permeability and inflammatory cell infiltration; it also promotes M2 macrophage polarization, indirectly suppressing TNF-α and LTB4 [41, 42]. Together, the drugs synergistically suppress inflammation through multi-targeted actions, leading to reduced cytokine levels and improved anti-inflammatory effects, which may contribute to improved prognosis.

In the pathological progression of GBM, VEGF-mediated pathological angiogenesis and blood-brain barrier disruption exacerbate cerebral edema and tumor growth; bFGF synergistically promotes angiogenesis, invasion, and microenvironment remodeling; TGF-β drives malignancy by suppressing immune function, inducing immunosuppression, and promoting EMT [43-45]. Our results indicate that the minocycline-bevacizumab combination was more effective than monotherapy in lowering serum levels of VEGF, bFGF, and TGF-β1 in GBM patients ($P < 0.05$), underscoring a multi-mechanistic synergy in suppressing tumor progression. This supports contemporary approaches that simultaneously target multiple pathways within the tumor microenvironment [46-48]. Bevacizumab mechanistically functions by directly neutralizing VEGF, thereby inhibiting abnormal angiogenesis and alleviating cerebral edema [16]. Minocycline complements this action by enhancing anti-angiogenic effects and suppressing tumor invasion through the inhibition of MMP activity and blockade of bFGF release and activation [27, 49]. Moreover, minocycline modulates the immune microenvironment, inhibits microglia/macrophage M2 polarization, reduces TGF-β1 secretion, and thereby reverses immunosuppression and enhances anti-tumor immunity [11, 50]. Together, the two drugs synergistically inhibit EMT and suppress malignant progression through multiple pathways.

The overall incidence of adverse events was comparable between groups ($P > 0.05$). A focused analysis of minocycline-associated toxicities - specifically skin pigmentation, hepatotoxicity, and headache - revealed low and statistically comparable incidences between the observation and control groups. No cases of intracranial hypertension or autoimmune hepatitis were confirmed. This manageable safety profile supports the feasibility of further clinical evaluation of the combination regimen.

The observation group exhibited significantly longer median PFS and OS, alongside greater

improvements in all quality-of-life domains compared to controls [51]. The concurrent enhancement of survival and patient-reported outcomes indicates comprehensive clinical benefit. This may be attributable to reduced symptom burden from delayed progression, preserved neurological function due to a tolerable safety profile, and psychological benefits derived from effective disease control, potentially mediated by reduced neuroinflammation [52]. These integrated benefits substantiate the strategy's further investigation in GBM management.

This multicenter study provides clinical and biomarker evidence supporting the potential synergy of bevacizumab and minocycline. However, this study also has some limitations. The retrospective design and the lack of comprehensive molecular profiling (e.g., MGMT, IDH) and standardized monitoring for specific bevacizumab-related toxicities (e.g., hypertension, proteinuria) introduce the possibility of unmeasured confounding. Consequently, we were unable to perform a multivariable Cox regression to report adjusted hazard ratios, and the survival benefits should be interpreted as exploratory. Furthermore, the safety analysis, while now including minocycline-specific events, could not fully account for all bevacizumab-associated adverse events due to inconsistent documentation. The minocycline dosing regimen and the comprehensive safety profile of the combination also require further validation in prospective settings. These limitations underscore the necessity for future randomized controlled trials that include prospective molecular profiling and systematic toxicity monitoring to confirm the efficacy and therapeutic value of this combination.

Conclusion

In conclusion, this retrospective analysis suggests that bevacizumab plus minocycline is associated with promising efficacy, including improved survival and quality of life, in GBM patients. Despite the inherent limitations, these data indicate potential synergy and justify future prospective, randomized trials to confirm the therapeutic value of this combination.

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Disclosure of conflict of interest

None.

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