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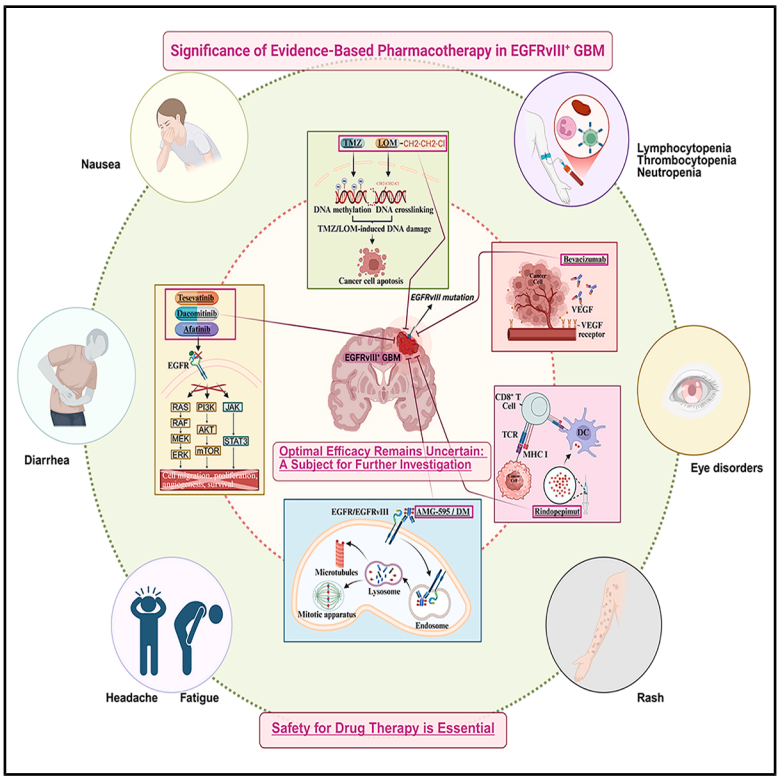
Comparative efficacy and safety of therapeutic strategies for EGFRvIII positive recurrent glioblastoma

Graphical abstract



Highlights

- Rind+Beva showed top OS, PFS, and ORR rankings in EGFRvIII$^+$ rGBM
- Rind+Beva showed lowest all grade and grade ≥3 AEs incidence in EGFRvIII$^+$ rGBM
- Targeted vaccines+anti-VEGF therapy may improve outcomes in EGFRvIII$^+$ rGBM
- More multicenter trials are needed to confirm Rind+Beva efficacy in EGFRvIII$^+$ rGBM

Authors

Jinming Qiu, Fangge Zhu, Rong Quan, Haoyuan Ye, Wenting Su, Renxi Wang, Yi Fu

Correspondence

renxi_wang@ccmu.edu.cn (R.W.), fuyi@ccmu.edu.cn (Y.F.)

In brief

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Comparative efficacy and safety of therapeutic strategies for EGFRvIII positive recurrent glioblastoma

Jinming Qiu,[1] Fangge Zhu,[1] Rong Quan,[2] Haoyuan Ye,[1] Wenting Su,[1] Renxi Wang,[1,*] and Yi Fu[1,3,*]
[1]Beijing Institute of Brain Disorders, Laboratory of Brain Disorders, Ministry of Science and Technology, Collaborative Innovation Center for Brain Disorders, Capital Medical University, No.10 Xitoutiao You'anmenwai Fengtai District, Beijing 100069, P.R. China
[2]Institute of Animal Husbandry and Veterinary Medicine, Beijing Academy of Agriculture and Forestry Sciences, No. 9 Shuguang Garden Middle Road Haidian District, Beijing 100097, P.R. China
[3]Lead contact
*Correspondence: renxi_wang@ccmu.edu.cn (R.W.), fuyi@ccmu.edu.cn (Y.F.)
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SUMMARY

Glioblastoma multiforme (GBM) is the most aggressive primary brain tumor, and EGFRvIII mutation has been associated with treatment resistance and poor prognosis, highlighting the need for more effective therapeutic strategies. We conducted a random-effects Bayesian network meta-analysis to compare the efficacy and safety of treatments for EGFRvIII-positive recurrent GBM (rGBM), evaluating overall survival (OS), progression-free survival (PFS), and objective response rate (ORR). Seven clinical trials were included (n = 716). Rindopepimut (Rind) + Bevacizumab (Beva) emerged as the most promising regimen, supported by its OS advantage in randomized trials, consistently favorable OS and PFS trends, top rankings in all three endpoints, and recommendation by clinical guidelines. Safety analysis showed that Rind + Beva had the lowest incidence of all-grade and grade ≥3 AEs. In conclusion, Rind + Beva represent the leading candidate for EGFRvIII-positive rGBM treatment, with the combination of molecular-targeted vaccines and anti-VEGF antibodies offering a promising strategy.

INTRODUCTION

Glioblastoma multiforme (GBM) is the most aggressive and highly heterogeneous primary brain malignancy, characterized by a poor prognosis. According to the Central Brain Tumor Registry of the United States (CBTRUS), the average annual incidence rate of malignant brain tumors in the United States between 2017 and 2021 was 6.89 per 100,000 individuals, with GBM accounting for 51.5% of all malignant brain tumors.[1] Due to its high molecular and histological heterogeneity, developing effective therapies for GBM remains a major challenge. Common genetic mutations found in GBM include IDH1/IDH2 mutations, ATRX mutations, TERT promoter mutations, NF1 inactivation, and EGFR amplification or deletion.[2] These genetic aberrations greatly influence tumor characteristics and the efficacy of anti-GBM therapies.

Among the various genetic mutations in GBM, EGFR variant III (EGFRvIII) is the most common EGFR mutation, occurring in 25%–30% of GBM patients. EGFRvIII features an in-frame deletion of exons 2–7, resulting in the loss of its ligand-binding domain, but the receptor still retains constitutive kinase activity.[3] Although the kinase activity of EGFRvIII is weaker than that of the full-length EGFR, it is still sufficient to drive tumorigenesis.[4] Studies have demonstrated that EGFRvIII plays a crucial role in GBM progression by promoting tumor initiation, enhancing invasiveness, increasing proliferation, and inhibiting apoptosis. EGFRvIII-positive cells secrete leukemia inhibitory factor (LIF) and IL-6, leading to gp130 activation and subsequent tumor growth promotion.[5] Additionally, some researchers suggest that EGFRvIII is predominantly expressed in brain cancer stem-like cells (bCSCs), which exhibit strong self-renewal capacity and enhanced resistance to anti-tumor therapies.[6]

Current treatment options for EGFRvIII-positive GBM mainly include EGFR small molecule inhibitors (tyrosine kinase inhibitors, TKIs), anti-EGFR antibodies, anti-EGFRvIII vaccines, and chimeric antigen receptor (CAR) T-cell therapies. EGFR-TKIs are designed to target GBM by binding to the tyrosine kinase domains of EGFR and other ERBB family members.[7] Clinical studies have shown that, compared with patients with EGFRvIII-negative tumors, those with EGFRvIII-positive tumors treated with the second-generation ErbB inhibitor afatinib have a longer median PFS (3.35 months vs. 0.99 months).[8] Conventional anti-EGFR antibodies target the L2 domain to block ligand binding and inhibit EGFR dimerization. However, due to the deletion mutation in the ligand-binding domain of EGFRvIII, these antibodies fail to exert their inhibitory effects.[7,9] An anti-EGFR antibody, mAb 806 (ABT-806), can selectively target mutant EGFRvIII and has shown superior antitumor activity in EGFRvIII-expressing tumors compared to cetuximab.[10] Moreover, this antibody can be conjugated with toxins or radioisotopes to enhance tumor cell killing.

Depatuxizumab mafodotin (Depatux-M/DM), an antibody-drug conjugate (ADC) composed of ABT-806 and the toxin monomethylauristatin-F, has demonstrated potential efficacy in rGBM with EGFRvIII mutations when combined with TMZ. Notably, Depatux-M appears to cf. a more pronounced OS advantage in newly diagnosed GBM (ndGBM) patients compared to those with rGBM.[11,12] Another ADC, AMG 595, exhibited favorable pharmacokinetics in a phase 1 trial and may benefit certain EGFRvIII-positive GBM patients with limited treatment options.[13] Anti-EGFRvIII targeted vaccines can activate the host immune system and elicit a durable immune response. Rindopepimut (Rind), also known as CDX-110, is a vaccine directed against EGFRvIII, composed of an EGFRvIII-specific peptide conjugated to keyhole limpet hemocyanin. Clinical studies have indicated that although CDX-110 + TMZ does not improve the median OS in ndGBM patients, CDX-110 + Bevacizumab (Beva) significantly enhances OS in patients with rGBM.[14,15] CAR T cell therapy utilizes engineered receptors that consist of a single-chain variable fragment (scFv) derived from a monoclonal antibody fused with transmembrane and intracellular activation domains, thereby enabling immune cells to selectively recognize and lyse tumor cells. Recent studies have shown that the combination of CAR T cells and PD-1 inhibitors in EGFRvIII-positive GBM is both safe and biologically active, and its clinical efficacy remains limited.[16]

In this study, we have undertaken a comprehensive systematic review and Bayesian network meta-analysis (NMA) to evaluate the efficacy and safety of various treatment strategies for EGFRvIII-positive GBM. By integrating data from multiple studies, we aim to provide a comparative assessment of OS, PFS, and objective response rate (ORR) among different therapeutic approaches. Additionally, we analyzed the incidence of adverse events (AEs) to offer insights into the risk-benefit profile of each treatment. Our findings will assist clinical decision-making and contribute to optimizing therapeutic strategies for patients with EGFRvIII-positive GBM.

RESULTS

Systematic review and characteristics of the included studies

A comprehensive literature search identified a total of 144 records from electronic databases, supplemented by 4 additional online records from conference proceedings. Following the removal of duplicates and an initial screening of abstracts for relevance, 24 studies were deemed eligible for full-text review. Among these, 9 studies focused on patients with newly diagnosed glioblastoma, and 15 studies targeted patients with recurrent glioblastoma. Due to insufficient data on EGFRvIII mutation in ndGBM patients,[11,15,17–22] finally 7 studies[8,12–14,23–25] focusing on rGBM patients met the eligibility criteria for inclusion in this analysis (Figure 1). The included studies collectively enrolled 716 patients, all of whom were histologically confirmed to be diagnosed with glioblastoma and generally met the 2021 WHO Classification of Tumors of the CNS. All of patients were assigned to one of the following 10 treatment regimens: Std. treatment, DM, DM + TMZ, AFA, AFA + TMZ, Beva, Rind + Beva, AMG 595, tesevatinib, and dacomitinib. Detailed characteristics of all included studies were provided in Tables 1, S5, and S6, while the network evidence diagrams were illustrated in Figures 2A and 3A.

Publication bias and evidence grade

The risk of bias (RoB) for the included studies was systematically assessed, with results displayed in the Figure S1. To evaluate potential publication bias, Egger's regression test was conducted, yielding a p value of 0.59 (Figure S2), which suggests no significant evidence of publication bias among the included studies. Based on the GRADE assessment tool, NMA evidence on OS and PFS for EGFRvIII-positive rGBM patients was moderate, and that on ORR for rGBM was low (Table S4).

NMA at OS for EGFRvIII mutation recurrent GBM patients

A total of 7 studies involving 5 distinct treatment regimens were analyzed, with each node in the network representing a unique treatment. The network included 5 direct comparisons. For patients with EGFRvIII-positive rGBM, Rind + Beva ranked as the most effective treatment based on SUCRA probabilities (SUCRA = 0.746), followed by DM + TMZ, (SUCRA = 0.718), DM (SUCRA = 0.450), Std. treatment, (SUCRA = 0.384), and Beva (SUCRA = 0.201) (Figures 2B and 2F). Notably, Rind + Beva demonstrated superior performance across multiple ranking measures, including the highest average rank, the greatest probability of being the best treatment, and the highest SUCRA score (Figures 2C and 2D). However, no statistically significant differences in OS were observed among the 5 regimens (Figure 2E). Rind + Beva's apparent advantage should be interpreted with caution in the absence of definitive statistical significance. A full summary of all pairwise comparisons is provided in Figure S9. The random-effects consistency model demonstrated excellent goodness-of-fit, with a deviance information criterion (DIC) of 7.977. Due to the limited number of direct and indirect comparisons, detailed heterogeneity between OS analyses could not be calculated.[26] In the global inconsistency assessment for OS outcomes, the DIC difference between the consistency and inconsistency models was only 0.0025 (Table S3). This negligible difference indicates that both models fit the data almost identically, thereby providing strong evidence that there is no inconsistency between the direct and indirect evidence within the network. These findings suggest that while Rind + Beva ranks highest, the lack of statistically significant differences across regimens highlight the need for further research to validate these results.

NMA at PFS for EGFRvIII mutation recurrent GBM patients

For patients with EGFRvIII-positive rGBM, seven treatment regimens were evaluated based on their PFS. Rind + Beva ranked as the most effective treatment, achieving the highest SUCRA score (SUCRA = 0.784), followed by DM + TMZ (SUCRA = 0.591), Beva (SUCRA = 0.526), AFA + TMZ (SUCRA = 0.523), Std. treatment, (SUCRA = 0.431), AFA (SUCRA = 0.375), and DM (SUCRA = 0.270) (Figures 3B and 3F). Rind + Beva demonstrated superior performance across multiple measures, including the highest average rank, the greatest probability of being the best treatment, and the top SUCRA score (Figures 3C and 3D). However, no statistically significant differences in PFS were observed

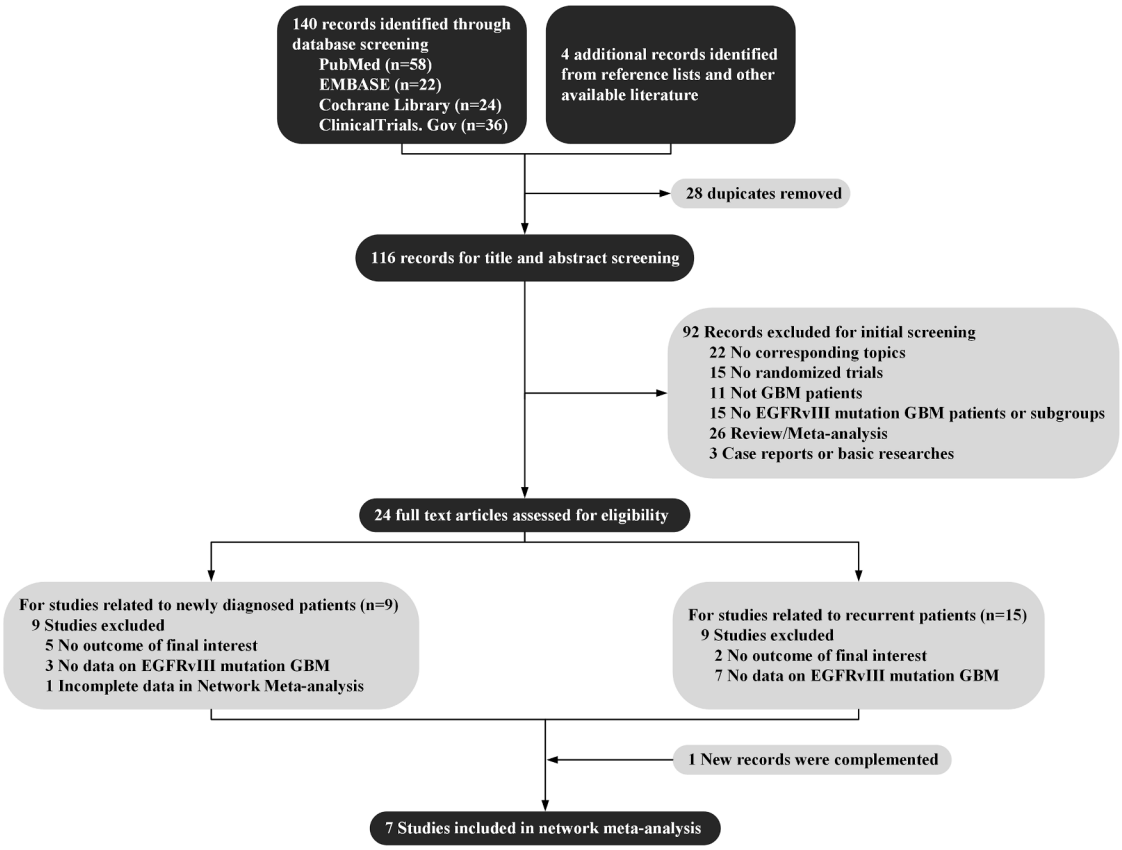


Figure 1. Literature search and selection

The study process followed the PRISMA guidelines. GBM, glioblastoma multiforme; EGFRvIII, EGFR variant III; PRISMA, preferred reporting items for systematic reviews and meta-analysis.

among the seven regimens (Figure 3E). Comprehensive results for all pairwise comparisons were detailed in Figure S10. The random-effects consistency model exhibited good fit, as indicated by a DIC of 11.957. Due to the limited number of direct and indirect comparisons available, detailed heterogeneity for PFS analyses could not be assessed.[26] For PFS outcomes, the global inconsistency test revealed a DIC difference of only 0.0135 between the consistency and inconsistency models (Table S3). This minimal discrepancy demonstrates that both models fit the data nearly identically, thereby providing strong evidence that there is no inconsistency between the direct and indirect evidence within the network. These findings suggest that while Rind + Beva consistently ranks highest in efficacy, the absence of significant differences across treatments underscores the need for further investigation.

NMA at ORR for EGFRvIII mutation recurrent GBM patients

For patients with EGFRvIII-positive rGBM, four treatment regimens were evaluated based on their ORR. Among the four therapies, Rind + Beva demonstrated the highest posterior density, indicating superior performance in ORR, with a median ‘posterior estimate of 30.67% (95% CIs: 20.83%–41.49%). Conversely, tesevatinib exhibited a relatively lower median posterior estimate of 15.35% (95% CIs: 2.08%–38.41%). AMG 595 and dacomitinib showed intermediate results, with median ORRs of 8.85% (95% CIs: 1.91%–20.28%) for AMG 595 and 9.50% (95% CIs: 1.22%–24.79%) for dacomitinib (Figures 4A and 4B). Meanwhile, Rind + Beva had the highest probability of being ranked first (96.06%), followed by tesevatinib (3.50%), dacomitinib (0.34%), and AMG 595 (0.10%). Conversely, tesevatinib had the highest probability of being ranked fourth (67.2%), suggesting its relatively lower efficacy in ORR (Figures 4C and 4E). Moreover, Rind + Beva was identified as the most efficacious treatment option, as reflected by its outstanding SUCRA score (SUCRA = 0.987), with tesevatinib (SUCRA = 0.415), AMG 595 (SUCRA = 0.346) and dacomitinib (SUCRA = 0.252) following (Figure 4D). The random-effects Bayesian model exhibited excellent fit, with a DIC value of 15.04.

Sensitivity assessment for OS and PFS

Due to the scarcity of bevacizumab-specific data in EGFRvIII-positive rGBM, a sensitivity analysis of the bevacizumab node was deemed necessary to assess the robustness of our NMA findings. Initially, we conducted a structural sensitivity analysis by merging the bevacizumab node with the standard treatment node. This modification enabled us to thoroughly examine the impact of alternative node definitions on the treatment rankings. Notably, even after the merger, the Rind + Beva treatment group

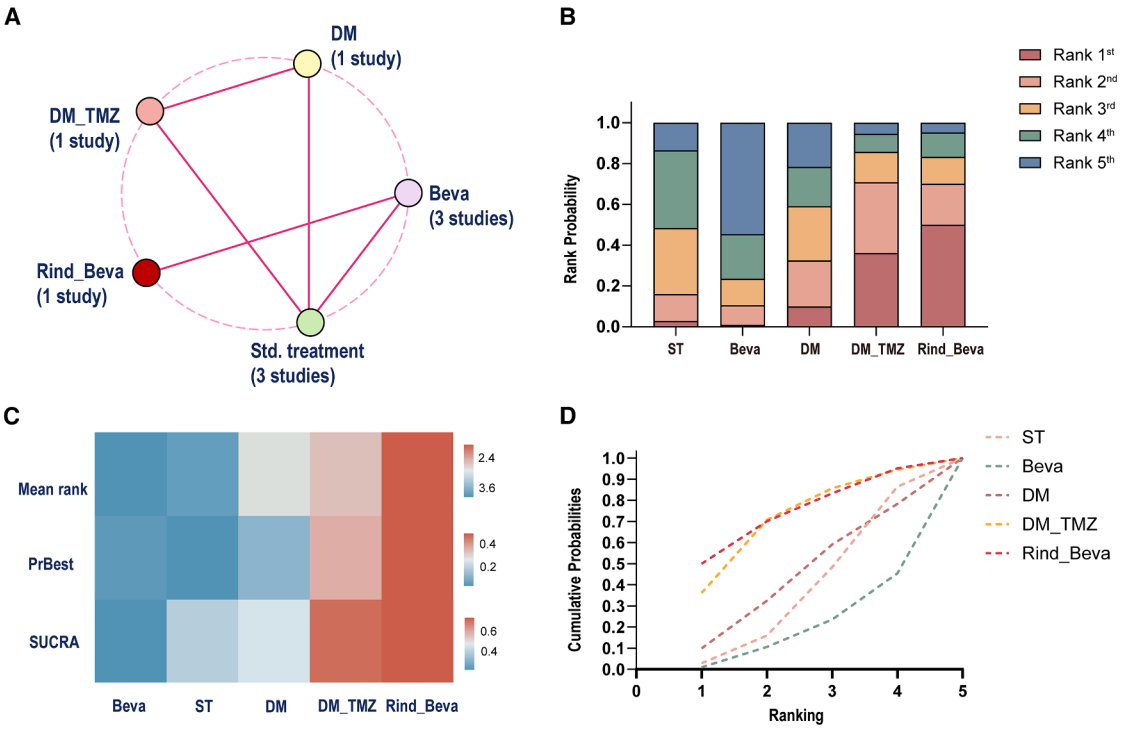
Table 1. Characteristics of included studies relating to EGFRvIII mutation positive glioblastoma

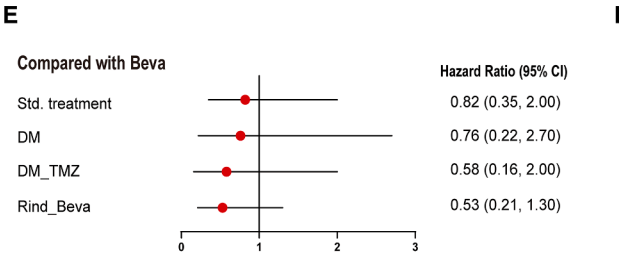
Study	Trial design	NO. of arms included in NMA	Patients number/ EGFRvIII mutation GBM	Total men (%)	Median age (yr, range)	Time since diagnosis of recurrence/ progression	Intervention and comparison	ORR of EGFRvIII mutation GBM	HR for OS of EGFRvIII mutation GBM	HR for PFS of EGFRvIII mutation GBM	Outcomes
Clinicaltrails. gov, 2021	Phase II NCT 02844439	1	40/11	8 (72.7%)	58.0 (37.0, 69.0)	0.80 (0.20, 2.70) months	Tesevatinib	9.1%	NA	NA	③④
Van den Bent, 2020	Phase II NCT 02343406	3	260/122	167 (64.2%)	TMZ or LOM 58.8 (34.9, 82.3) DM Mono 58.3 (36.3, 79.3) DM + TMZ 59.2 (40.1, 75.4)	TMZ or LOM 6.23 ± 4.56 weeks DM Mono 5.81 ± 3.31 weeks DM + TMZ 6.03 ± 4.30 weeks	TMZ or LOM DM Mono DM + TMZ	NA	DM + TMZ 0.70 (0.43, 1.13) DM Mono 0.93 (0.57, 1.52)	DM + TMZ 0.87 (0.54, 1.40) DM Mono 1.22 (0.73, 2.04)	①②④ ⑤⑥⑦
Reardon, 2020	Phase II NCT 01498328	2	73/73	41 (56.2%)	Rind+Beva 59.0 (44.0, 79.0) Beva Mono 55.0 (30.0, 75.0)	Beva Mono 11.60 (4.70, 38.30) months Rind+Beva 10.80 (3.70, 55.20) months	Beva Mono Rind+Beva	30%	0.53 (0.32, 0.88)	0.72 (0.43, 1.21)	①②③④
Rosenthal, 2019	Phase I NCT 01475006	1	32/32	24 (75.0%)	57.0 (39.0, 73.0)	12.20 (4.90–34.30) months	AMG 595	6%	NA	NA	③④
Sepúlveda-Sánchez, 2017	Phase II NCT 01520870	1	49/19	32 (65.3%)	59.0 (39.0, 81.0)	NA	Dacomitinib	5.3%	NA	NA	①②③④ ⑤⑥⑦
Reardon, 2015	Phase II NCT 00727506	3	119/51	73 (61.3%)	TMZ 56.9 ± 10.62 AFA Mono 56.6 ± 9.44 AFA + TMZ 55.4 ± 11.02	TMZ 9.20 (3.60, 70.60) months Afatinib Mono 11.70 (4.00, 57.80) months Afatinib+TMZ 11.00 (4.60, 122.80) months	TMZ AFA Mono AFA +TMZ	NA	NA	AFA mono 1.19 (0.30, 4.79) AFA + TMZ 0.90 (0.24, 3.40)	②④⑤⑥⑦
Taal, 2014; Cochrane Library, 2021[a]	Phase II NTR 1929	2	153/NA	91 (59.5%)	LOM 56.0 (28.0, 73.0) Beva Mono 58.0 (37.0, 77.0)	NA	LOM Beva Mono	NA	1.22 (0.84, 1.76)[a]	0.90 (0.58, 1.38)[a]	⑤⑥⑦

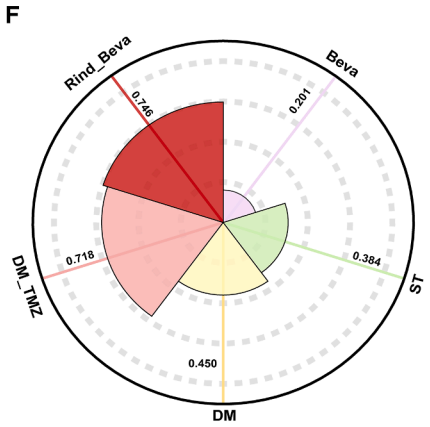
Outcomes: ①, OS for EGFRvIII mutation GBM; ②, PFS for EGFRvIII mutation GBM; ③, ORR for EGFRvIII mutation GBM; ④, AEs for EGFRvIII mutation GBM; ⑤, OS for total patients; ⑥, PFS for total patients; ⑦, AEs for total patients.

TMZ, temozolomide; LOM, lomustine; Beva, bevacizumab; Rind, rindopepimut; DM, Depatux-M; Afatinib, AFA; Mono, Monotherapy; HR, hazard ratio; OS, overall survival; PFS, progression free survival; GBM, glioblastoma; NA, not available.

[a]HR on Beva mono result was calculated by pooling other results together.







G

Rank 5th				
SUCRA= 0.201	Rank 4th			
Beva	SUCRA= 0.384	Rank 3rd		
1.20 (0.52, 2.90)	Std. treatment	SUCRA= 0.450	Rank 2nd	
1.30 (0.37, 4.70)	1.10 (0.43, 2.60)	DM	SUCRA= 0.718	Rank 1st
1.70 (0.50, 6.10)	1.40 (0.58, 3.50)	1.30 (0.53, 3.30)	DM_TMZ	SUCRA= 0.746
1.90 (0.75, 4.70)	1.50 (0.44,5.50)	1.40 (0.31, 6.80)	1.10 (0.23, 5.10)	Rind_Beva

Figure 2. Results of OS for EGFRvIII mutation recurrent glioblastoma

(A) Network plot for 5 available treatments.

(B) Stacked chart displaying the ranking of five treatments. It illustrates the distribution of rankings (from 1st to 5th) across five different treatment options.

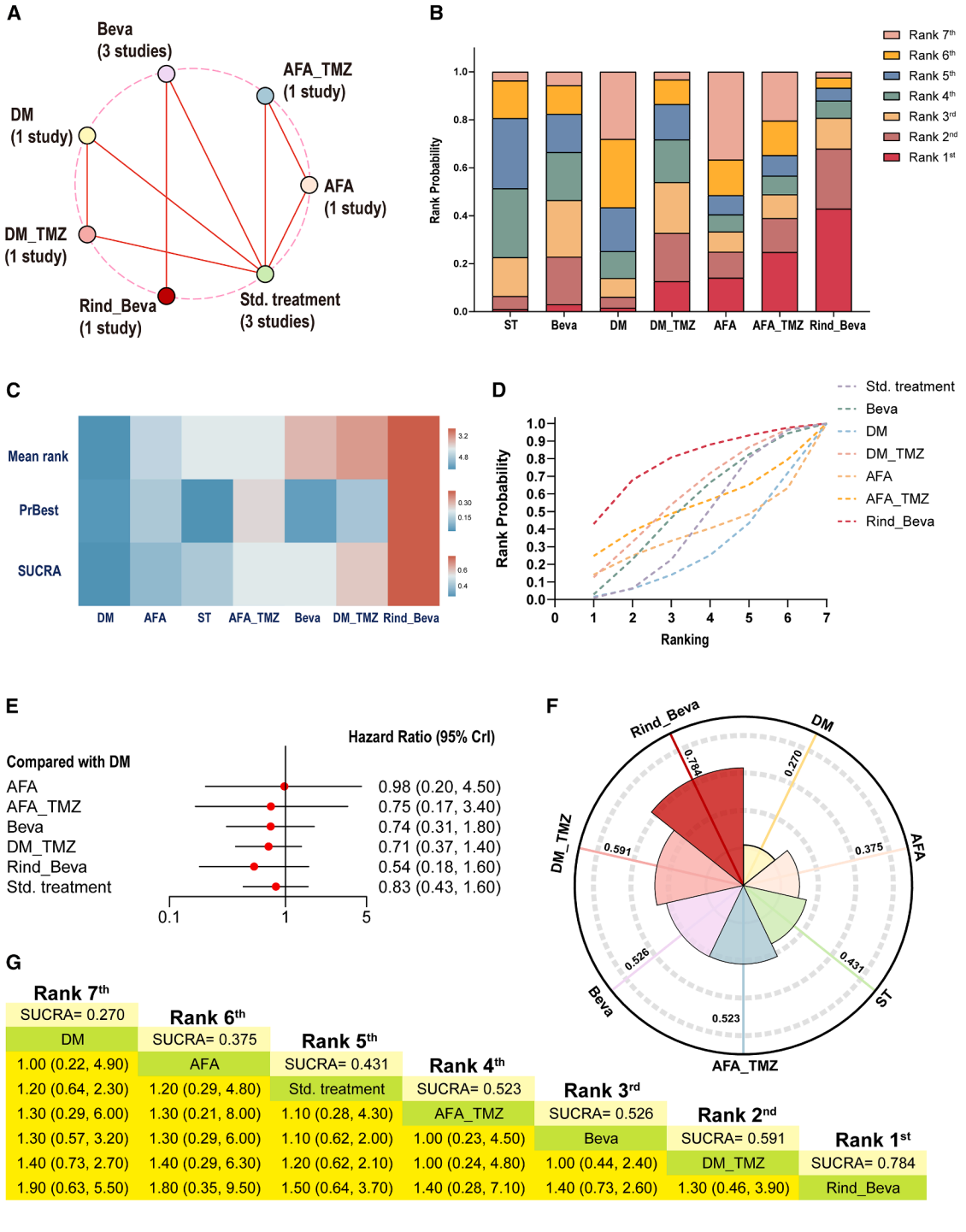
(C) Heatmap of mean rank, PrBest, and SUCRA values for five treatments. Lower mean rank values denote better performance, whereas higher PrBest and SUCRA values imply a greater likelihood of being the most effective treatment.

(D) Cumulative ranking probability curves for five treatments. Higher curves indicate a greater probability of achieving superior ranks, reflecting higher treatment efficacy.

(E) Forest plot of the other top 4 treatments compared to Std. treatment. Data are represented as mean ± SEM.

(F) Radar diagram for SUCRA results of 5 treatments.

(G) Comparisons between each treatment (Hazard ratios and 95% CIs for overall survival). Each treatment comparison is represented by a yellow cell displaying the HR and its 95% CI that compares the column treatment to the orange row treatment. Data are represented as mean ± SEM. ST/Std. treatment, standard treatment; Beva, bevacizumab; DM, Depatux-M; TMZ, temozolomide; Rind, rindopepimut; OS, overall survival; HR, hazard ratio; CI, confidence interval; SUCRA, surface under the cumulative ranking curve; PrBest, probability of being the best.



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Rank 7th	Rank 6th	Rank 5th	Rank 4th	Rank 3rd	Rank 2nd	Rank 1st
SUCRA= 0.270	SUCRA= 0.375	SUCRA= 0.431	SUCRA= 0.523	SUCRA= 0.526	SUCRA= 0.591	SUCRA= 0.784
DM	AFA	Std. treatment	AFA_TMZ	Beva	DM_TMZ	Rind_Beva
1.00 (0.22, 4.90)	1.20 (0.29, 4.80)	1.10 (0.28, 4.30)	1.00 (0.23, 4.50)	1.00 (0.44, 2.40)	1.30 (0.46, 3.90)	
1.20 (0.64, 2.30)	1.30 (0.21, 8.00)	1.10 (0.62, 2.00)	1.00 (0.24, 4.80)	1.40 (0.73, 2.60)		
1.30 (0.29, 6.00)	1.30 (0.29, 6.00)	1.20 (0.62, 2.10)	1.40 (0.28, 7.10)			
1.30 (0.57, 3.20)	1.40 (0.29, 6.30)	1.50 (0.64, 3.70)				
1.40 (0.73, 2.70)	1.80 (0.35, 9.50)					
1.90 (0.63, 5.50)						

Figure 3. Results of PFS for EGFRvIII mutation recurrent glioblastoma

(A) Network plot for 7 available treatments.

(B) Stacked chart displaying the ranking of seven treatments. It illustrates the distribution of rankings (from 1st to 7th) across seven different treatment options.

(C) Heatmap of mean rank, PrBest, and SUCRA values for seven treatments. Lower mean rank values denote better performance, whereas higher PrBest and SUCRA values imply a greater likelihood of being the most effective treatment.

(D) Cumulative ranking probability curves for seven treatments. Higher curves indicate a greater probability of achieving superior ranks, reflecting higher treatment efficacy.

(E) Forest plot of the other top 6 treatments compared to Std. treatment. Data are represented as mean ± SEM.

(F) Radar diagram for SUCRA results of 7 treatments.

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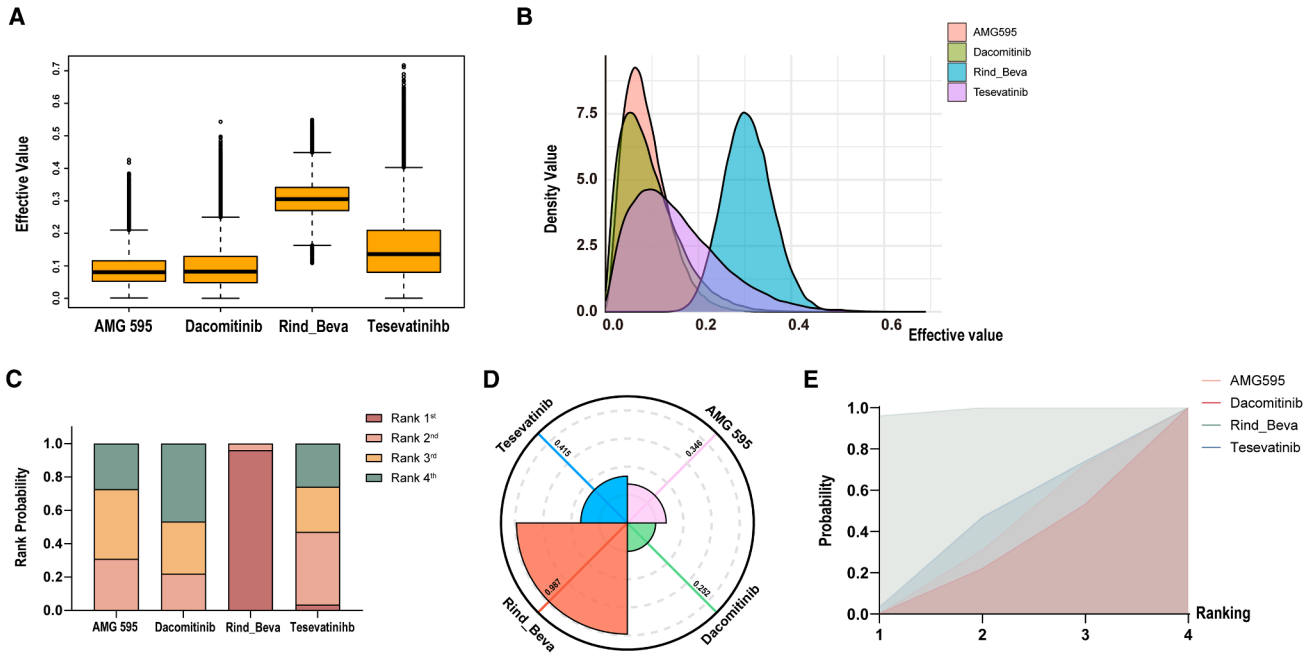

Figure 4. Results of ORR for EGFRvIII mutation recurrent glioblastoma

(A) Boxplots illustrating the posterior distributions of ORR effect sizes across four different treatment groups.

(B) Posterior distribution of the overall ORR effect size.

(C) Stacked chart displaying the ranking of four treatments. It illustrates the distribution of rankings (from 1st to 4th) across four different treatment options.

(D) Radar diagram for SUCRA results of 4 treatments.

(E) Bayesian ranking profiles for four different drugs. Higher surface under the cumulative ranking curve implies a greater likelihood of being the most effective treatment. Rind, rindopepimut; Beva, bevacizumab; ORR, objective response rate.

retained its top rank (Figure S11), thereby attesting to the robustness of the treatment hierarchy with respect to network structure alterations. Subsequently, a parameter sensitivity analysis was performed by varying the OS and PFS estimates for the bevacizumab node by ±10%. The resulting shifts in treatment rankings were minimal, with the Rind + Beva group consistently emerging as the highest-ranked intervention (Figure S12). This outcome further confirms the stability and reliability of our primary findings. In summary, the convergence of evidence from both the structural and parameter sensitivity analyses unequivocally supports our principal conclusion: in the treatment of EGFRvIII-positive rGBM, the Rind + Beva regimen demonstrates a significant therapeutic advantage, with its superior ranking remaining robust across a range of model specifications.

Assessment of heterogeneity among included studies

The potential sources of heterogeneity in our study include: (1) variability in dosing and treatment schedules of standard therapies across trials; (2) differences in patient enrollment numbers; (3) divergent tumor molecular profiles among rGBM cohorts; and (4) heterogeneity in EGFRvIII detection methods. Firstly, as summarized in Table S8, the two bevacizumab trials employed identical dosing regimens—10 mg/kg administered intravenously every two weeks.[14,24] Among the three standard-therapy studies, lomustine dosing was also uniform at 110 mg/m^2 given orally every six weeks. In contrast, temozolomide regimens varied: Van den Bent et al. administered 150–200 mg/m^2 on days 1–5 of a 28-day cycle, whereas Reardon et al. used 75 mg/m^2 on days 1–21 of a 28-day cycle.[8,12,24] Different dosing schedules may influence both efficacy and toxicity outcomes, potentially introducing confounding effects when comparing treatment arms that include TMZ. This heterogeneity may reduce the comparability of these studies and bias the pooled estimates for standard treatment arms. Moreover, as shown in Table S1, only the Van den Bent et al. study enrolled over 100 EGFRvIII-positive patients.[12] The other trials included much smaller cohorts,[8,13,14,23–25] and Reardon et al. (2020) specifically acknowledged their limited sample size as a key study constraint.[14] Small studies tend to have wider confidence intervals and greater statistical uncertainty, which could increase variability in effect estimates and reduce the overall precision of the NMA. Additionally, in Table S5 we observed that three studies enrolled EGFRvIII-positive patients with concurrent EGFR amplification,[12,23,25] while one study included patients exhibiting a combined gain of

(G) Comparisons between each treatment (Hazard ratios and 95% CIs for overall survival). Each treatment comparison is represented by a yellow cell displaying the HR and its 95% CI that compares the column treatment to the orange row treatment. Data are represented as mean ± SEM. ST/Std. treatment, standard treatment; Beva, bevacizumab; AFA, afatinib; DM, Depatux-M; TMZ, temozolomide; Rind, rindopepimut; PFS, progression-free survival. HR, hazard ratio; CI, confidence interval; SUCRA, surface under the cumulative ranking curve; PrBest, probability of being the best.

A

Adverse effect	General disorders						Gastrointestinal disorders	
Intervention	Fatigue	Musculoskeletal	Arthralgia	Pain	Decreased appetite	Other General disorders	Diarrhea	Nausea/Vomiting
Rind_Beva	8/35 (22.86%)	NA	8/35 (22.86%)	6/35 (17.14%)	NA	NA	6/35 (17.14%)	15/35 (42.86%)
DM_TMZ	33/88 (37.50%)	27/88 (30.68%)	NA	NA	NA	NA	8/88 (9.09%)	21/88 (23.86%)
Afatinib_TMZ	NA	NA	NA	NA	1/39 (2.56%)	NA	32/39 (82.05%)	23/39 (58.97%)
DM	28/84 (33.33%)	16/84 (19.05%)	NA	NA	NA	NA	6/84 (7.14%)	9/84 (10.71%)
AMG 595	8/32 (25.00%)	NA	NA	NA	NA	NA	NA	6/32 (18.75%)
Afatinib	NA	NA	NA	NA	NA	NA	29/41 (70.73%)	10/41 (24.39%)
Dacomitinib	NA	NA	NA	NA	NA	11/49 (22.45%)	33/49 (67.35%)	4/49 (8.16%)
Tesevatinib	5/40 (12.50%)	NA	NA	1/40 (2.50%)	2/40 (5.00%)	4/40 (10.00%)	11/40 (27.50%)	2/40 (5.00%)
Bevacizumab	42/87 (48.28%)	NA	2/87 (2.30%)	3/87 (3.45%)	NA	NA	2/87 (2.30%)	12/87 (13.79%)
Std. treatment	41/162 (25.31%)	16/162 (9.88%)	NA	NA	NA	NA	8/162 (4.94%)	36/162 (22.22%)

Adverse effect	Metabolism and nutrition disorders					Infections and infestations	Vascular disorders	
Intervention	ALT increase	Bilirubin	Glucose	Hyperglycemia	Other metabolism and nutrition disorders		Venous thrombosis	Hypertension
Rind_Beva	NA	NA	NA	3/35 (8.57%)	NA	NA	NA	8/35 (2.29%)
DM_TMZ	50/88 (56.82%)	11/88 (12.50%)	3/88 (3.41%)	NA	NA	30/88 (34.09%)	1/88 (1.14%)	NA
Afatinib_TMZ	NA	NA	NA	NA	NA	NA	NA	NA
DM	34/84 (40.48%)	6/84 (7.14%)	3/84 (3.57%)	NA	NA	22/84 (26.19%)	1/84 (1.19%)	NA
AMG 595	6/32 (18.75%)	NA	NA	NA	8/32 (25.00%)	NA	NA	NA
Afatinib	NA	NA	NA	NA	NA	NA	NA	NA
Dacomitinib	NA	NA	NA	NA	NA	NA	NA	NA
Tesevatinib	NA	NA	NA	1/40 (2.50%)	3/40 (7.50%)	NA	1/40 (2.50%)	1/40 (2.50%)
Bevacizumab	NA	NA	NA	4/87 (4.60%)	NA	10/87 (11.49%)	NA	37/87 (42.53%)
Std. treatment	27/162 (16.67%)	5/162 (3.09%)	2/162 (1.23%)	NA	NA	19/162 (11.73%)	3/162 (1.85%)	11/162 (6.79%)

Adverse effect	Hematological disorders					Eye disorders	Respiratory, thoracic and mediastinal disorders	Injury, poisoning and procedural complications
Intervention	Hemoglobin	Neutrophils	Lymphocytes	Platelets	Purpura			
Rind_Beva	NA	NA	NA	NA	NA	NA	NA	6/35 (17.14%)
DM_TMZ	30/88 (34.10%)	19/88 (21.59%)	61/88 (69.32%)	61/88 (69.32%)	NA	73/88 (82.95%)	24/88 (27.27%)	NA
Afatinib_TMZ	NA	NA	NA	NA	NA	NA	NA	NA
DM	25/84 (29.76%)	6/84 (7.14%)	40/84 (47.62%)	NA	NA	60/84 (71.43%)	6/84 (7.14%)	NA
AMG 595	NA	NA	NA	16/32 (50.00%)	NA	NA	NA	NA
Afatinib	NA	NA	NA	NA	NA	NA	NA	NA
Dacomitinib	NA	NA	NA	NA	NA	NA	NA	NA
Tesevatinib	NA	NA	1/40 (2.50%)	NA	NA	2/40 (5.00%)	2/40 (5.00%)	2/40 (5.00%)
Bevacizumab	NA	NA	NA	50/87 (57.47%)	15/87 (17.24%)	NA	7/87 (8.05%)	2/87 (2.30%)
Std. treatment	50/162 (30.86%)	41/162 (25.31%)	51/162 (31.48%)	116/162 (71.60%)	11/162 (6.79%)	3/162 (1.85%)	13/162 (8.02%)	NA

Adverse effect	Nervous system disorders					Skin and subcutaneous tissue disorders		
Intervention	Convulsion	Headache	Hemiparesis	Other nervous system disorders	Any	Rash	Dermatitis acneiform	Rash papular
Rind_Beva	8/35 (22.86%)	8/35 (22.86%)	2/35 (5.71%)	NA	NA	NA	NA	NA
DM_TMZ	NA	NA	NA	NA	57/88 (64.77%)	7/88 (7.95%)	NA	NA
Afatinib_TMZ	NA	NA	1/39 (2.56%)	2/39 (5.13%)	NA	22/39 (56.41%)	4/39 (10.26%)	NA
DM	NA	NA	NA	NA	58/84 (69.05%)	2/84 (2.38%)	NA	NA
AMG 595	NA	NA	NA	NA	NA	NA	NA	NA
Afatinib	NA	1/41 (2.44%)	NA	5/41 (12.20%)	NA	19/41 (46.34%)	NA	NA
Dacomitinib	NA	NA	NA	NA	NA	40/49 (81.63%)	NA	NA
Tesevatinib	NA	2/40 (5.00%)	NA	4/40 (10.00%)	NA	4/40 (10.00%)	5/40 (12.50%)	1/40 (2.50%)
Bevacizumab	9/87 (10.34%)	9/87 (10.34%)	6/87 (6.90%)	NA	NA	NA	NA	NA
Std. treatment	NA	NA	2/162 (1.23%)	NA	43/162 (26.54%)	6/162 (3.70%)	NA	NA

B

Adverse effect	General disorders						Gastrointestinal disorders	
Intervention	Fatigue	Musculoskeletal	Arthralgia	Pain	Decreased appetite	Other General disorders	Diarrhea	Nausea/Vomiting
Rind_Beva	NA	NA	NA	2/35 (5.71%)	NA	NA	NA	NA
DM_TMZ	7/88 (7.95%)	2/88 (2.27%)	NA	NA	NA	NA	NA	NA
Afatinib_TMZ	2/39 (5.13%)	NA	NA	NA	NA	NA	3/39 (7.69%)	NA
DM	4/84 (4.76%)	3/84 (3.57%)	NA	NA	NA	NA	NA	1/84 (1.19%)
AMG 595	2/32 (6.25%)	NA	NA	NA	NA	NA	NA	2/32 (6.25%)
Afatinib	5/41 (12.19%)	NA	NA	NA	2/41 (4.88%)	NA	3/41 (7.31%)	NA
Dacomitinib	NA	NA	NA	NA	NA	2/49 (4.08%)	3/49 (6.12%)	NA
Tesevatinib	NA	NA	NA	NA	NA	NA	NA	NA
Bevacizumab	4/87 (4.60%)	NA	1/87 (1.15%)	NA	NA	NA	NA	2/87 (2.30%)
Std. treatment	7/162 (4.32%)	4/162 (2.47%)	NA	NA	NA	NA	NA	2/162 (1.23%)

Adverse effect	Metabolism and nutrition disorders					Infections and infestations	Vascular disorders	
Intervention	ALT increase	Bilirubin	Glucose	Hyperglycemia	Other metabolism and nutrition disorders		Venous thrombosis	Hypertension
Rind_Beva	NA	NA	NA	NA	NA	NA	NA	1/35 (2.86%)
DM_TMZ	1/88 (1.14%)	3/88 (3.41%)	NA	NA	NA	5/88 (5.68%)	NA	NA
Afatinib_TMZ	NA	NA	NA	NA	NA	NA	NA	NA
DM	1/84 (1.19%)	NA	NA	NA	NA	4/84 (4.76%)	NA	NA
AMG 595	1/32 (3.13%)	NA	NA	NA	1/32 (3.13%)	NA	NA	NA
Afatinib	NA	NA	NA	NA	NA	NA	NA	NA
Dacomitinib	NA	NA	NA	NA	NA	NA	NA	NA
Tesevatinib	NA	NA	NA	NA	NA	NA	NA	NA
Bevacizumab	NA	NA	NA	3/87 (3.45%)	NA	3/87 (3.45%)	NA	16/87 (18.39%)
Std. treatment	2/162 (1.23%)	NA	NA	NA	NA	6/162 (3.70%)	2/162 (1.23%)	3/162 (1.85%)

Adverse effect	Hematological disorders					Eye disorders	Respiratory, thoracic and mediastinal disorders	Injury, poisoning and procedural complications
Intervention	Hemoglobin	Neutrophils	Lymphocytes	Platelets	Purpura			
Rind_Beva	NA	NA	NA	NA	NA	NA	NA	1/35 (2.86%)
DM_TMZ	3/88 (3.41%)	5/88 (5.68%)	26/88 (26.95%)	NA	NA	29/88 (32.95%)	9/88 (10.23%)	NA
Afatinib_TMZ	NA	NA	NA	NA	NA	NA	NA	NA
DM	1/84 (1.19%)	1/84 (1.19%)	11/84 (13.10%)	NA	NA	20/84 (23.81%)	NA	NA
AMG 595	NA	1/32 (3.13%)	NA	15/32 (46.88%)	1/32 (3.13%)	NA	NA	NA
Afatinib	NA	NA	NA	1/41 (2.44%)	NA	NA	NA	NA
Dacomitinib	NA	NA	NA	NA	NA	NA	NA	NA
Tesevatinib	NA	NA	NA	NA	NA	NA	NA	NA
Bevacizumab	NA	NA	NA	1/87 (1.15%)	NA	NA	2/87 (2.30%)	1/87 (1.15%)
Std. treatment	8/162 (4.94%)	21/162 (12.96%)	18/162 (11.11%)	9/162 (5.56%)	NA	NA	3/162 (1.85%)	NA

Adverse effect	Nervous system disorders					Skin and subcutaneous tissue disorders		
Intervention	Convulsion	Headache	Hemiparesis	Other nervous system disorders	Any	Rash	Dermatitis acneiform	Rash papular
Rind_Beva	4/35 (11.42%)	1/35 (2.86%)	NA	NA	NA	NA	NA	NA
DM_TMZ	NA	NA	NA	NA	21/88 (23.86%)	NA	NA	NA
Afatinib_TMZ	NA	NA	NA	NA	NA	NA	3/39 (7.69%)	NA
DM	NA	NA	NA	NA	21/84 (25.00%)	NA	NA	NA
AMG 595	NA	NA	NA	1/32 (3.13%)	NA	NA	NA	NA
Afatinib	NA	NA	NA	NA	NA	NA	4/41 (9.76%)	NA
Dacomitinib	NA	NA	NA	NA	NA	11/49 (22.45%)	NA	NA
Tesevatinib	NA	NA	NA	2/40 (5.00%)	NA	NA	NA	NA
Bevacizumab	NA	2/87 (2.30%)	2/87 (2.30%)	NA	NA	NA	NA	NA
Std. treatment	NA	NA	NA	NA	15/162 (9.26%)	NA	NA	NA

(legend on next page)

chromosome 7 and loss of chromosome 10 alongside EGFRvIII.[8] These co-occurring genetic alterations may influence both treatment responsiveness and prognosis, acting as unmeasured confounders and potentially modifying the effect of targeted therapies. Finally, according to Table S8, EGFRvIII detection methods varied: one study[8] used immunohistochemistry (IHC), three[12,14,23] employed qPCR, and two[13,25] utilized both techniques. Differences in detection methods may lead to variability in patient classification. For example, IHC may detect protein-level expression, while qPCR assesses transcript levels. Inconsistencies in EGFRvIII identification may introduce misclassification bias and dilute treatment effects specific to this molecular subtype.

Based on the previous analysis, we noted that the study by Reardon et al. (2015) differed from the others in several aspects, including sample size, TMZ regimen, molecular characteristics, and EGFRvIII detection methods.[8] To assess its impact on the overall conclusions, we conducted a sensitivity analysis based on heterogeneity by excluding this study and reconstructing the network model. The results showed that the main effect estimates remained largely consistent with the original analysis, suggesting a certain degree of robustness (Figure S13). However, the findings should still be interpreted with caution.

Analysis of adverse events

Since the seven included studies provided only subgroup results for EGFRvIII-positive rGBM patients and lacked specific data on adverse drug reactions in this subgroup, we analyzed the overall safety profiles of the treatments in the broader GBM population. This approach enabled a more comprehensive understanding of the potential adverse effects associated with therapies targeting EGFRvIII-positive patients. By evaluating the AE data from the overall GBM cohort, we aimed to provide insights into the safety considerations that may influence treatment decisions for this specific molecularly defined subgroup.

Among all-grade AEs, gastrointestinal disorders were the most prevalent, occurring with all treatments. Specifically, 135 out of 625 patients (21.6%) experienced diarrhea, while 138 out of 657 patients (21.0%) reported nausea or vomiting. Thrombocytopenia was the most frequent AE overall, affecting 243 out of 369 patients (65.9%). Fatigue was the most common symptom among general disorders, occurring in 165 out of 528 patients (31.3%). For metabolism and nutrition disorders, ALT increase was the most frequent biochemical abnormality, noted in 87 out of 366 patients (23.8%). Hypertension was the leading vascular disorder, affecting 57 out of 324 patients (17.6%). In the nervous system disorders category, headache was the most frequently reported symptom, affecting 20 out of 203 patients (9.9%). Rash was the most common symptom in skin and subcutaneous tissue disorders, occurring in 38 out of 415 patients (9.2%). Additionally, infections and infestations affected 81 out of 421 patients (19.2%), while eye disorders were reported in 138 out of 374 patients (36.9%). Respiratory, thoracic, and mediastinal disorders were noted in 52 out of 461 patients (11.3%), and injury, poisoning, and procedural complications occurred in 10 out of 162 patients (6.2%) (Figure 5A). For grade $\geq$3 AEs, fatigue was the most frequent event, occurring in 31 out of 533 patients (5.8%). Eye disorders had the highest proportional occurrence among grade $\geq$3 AEs, affecting 49 out of 172 patients (28.5%). Diarrhea was the most prevalent symptom within gastrointestinal disorders, reported in 9 out of 129 patients (7.0%). Hyperglycemia was the most frequent biochemical abnormality in metabolism and nutrition disorders, occurring in 3 out of 87 patients (3.5%). Hypertension was the most common vascular disorder, affecting 20 out of 284 patients (7.0%). Lymphocytopenia was the most frequent hematological disorder, noted in 55 out of 334 patients (16.5%). Convulsion was the leading symptom among nervous system disorders, affecting 4 out of 35 patients (11.42%). Rash was the most common symptom in skin and subcutaneous tissue disorders, occurring in 11 out of 49 patients (22.45%). Additionally, infections and infestations affected 18 out of 421 patients (4.3%), while respiratory, thoracic, and mediastinal disorders were reported in 14 out of 337 patients (4.2%). Injury, poisoning, and procedural complications occurred in 2 out of 162 patients (1.6%) (Figure 5B).

In the analysis of individual treatments, notable AEs were observed across different regimens. A high proportion of patients receiving DM monotherapy (71.4%) and DM + TMZ (83.0%) experienced eye disorders while lymphocytopenia and thrombocytopenia were reported in 69.3% of patients receiving DM + TMZ combination therapy. Among patients treated with TMZ and Afatinib, 82.1% of patients experienced diarrhea, while 60.0% reported nausea/vomiting. Thrombocytopenia was frequently observed in 71.6% of patients treated with TMZ/LOM, 56.0% with bevacizumab, and 50.0% with AMG 595. In contrast, Rind + Beva exhibited a relatively better safety profile, with only 42.9% of patients experiencing nausea/vomiting, and no other major AEs reported. Skin-related adverse effects such as rash were more prominent in afatinib (56.4%) and dacomitinib (81.6%). For grade $\geq$3 AEs, lymphocytopenia was reported in 27.0% of patients receiving DM + TMZ and in 46.9% of those treated with AMG 595. Similarly, grade $\geq$3 eye disorders were observed in 23.8% of patients treated with DM monotherapy and 27.8% of those receiving DM + TMZ combination therapy. Additionally, 22.5% of patients treated with dacomitinib reported grade $\geq$3 rash. These findings suggest that Rind + Beva may offer a more tolerable safety profile compared to other regiments, with a lower incidence of common AEs such as nausea/vomiting and no significant occurrences of severe toxicities. This makes Rind + Beva a more attractive treatment option for EGFRvIII-positive rGBM patients.

Figure 5. Frequency of toxicity distribution associated with the incidence of each adverse event in a population based on multiple treatment regimens included for all recurrent GBM patients

(A) The frequency distribution of all grade adverse events based on treatment regimens.

(B) The frequency distribution of $\geq$3 grade adverse events based on treatment regimens. Cells in red, frequency $\geq$50%; in yellow, 20%–50%; in light green, 5%–20%; in dark green, <5%. NA, not available; Std. treatment, standard treatment; Beva, bevacizumab; DM, Depatux-M; TMZ, temozolomide; Rind, rindopepimut.

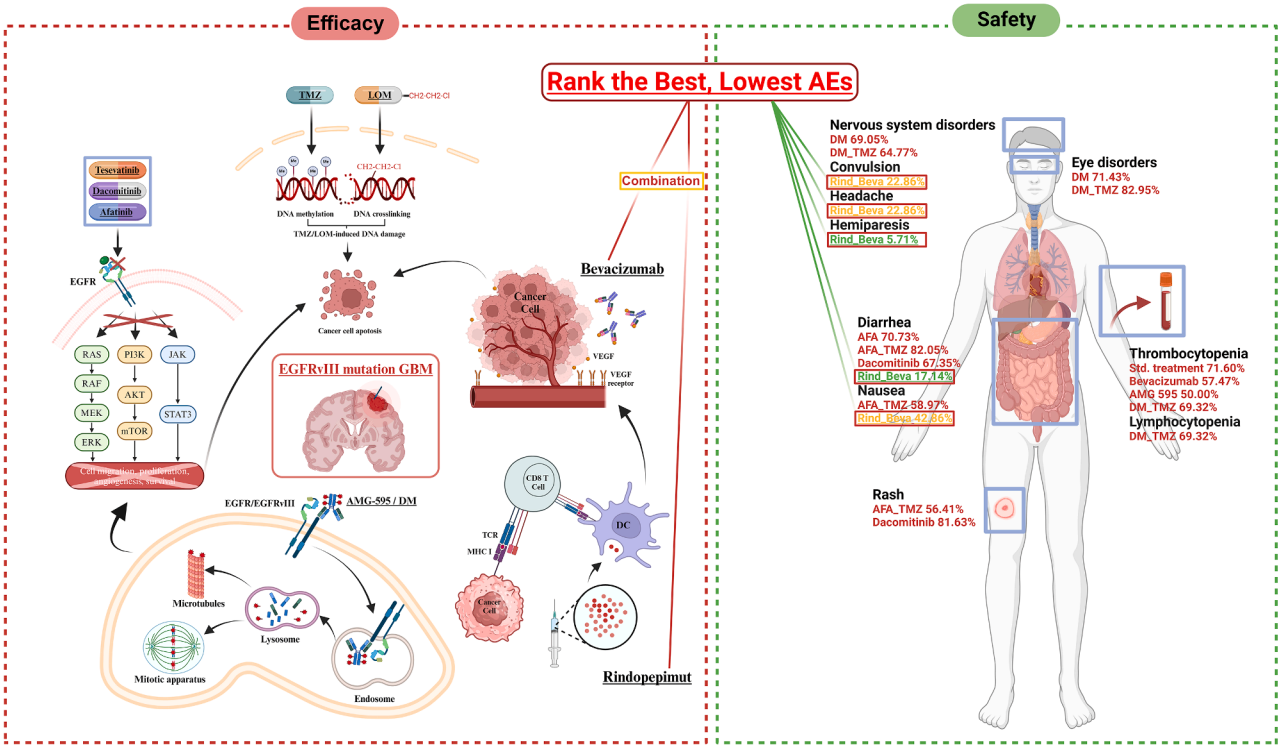

Figure 6. Summarized findings of current Bayesian analysis

Rindopepimut is an EGFRvIII-specific vaccine designed to elicit a targeted immune response against tumor cells harboring the mutation. Bevacizumab, a monoclonal antibody that inhibits angiogenesis, disrupts tumor vascularization. Combined, these agents may synergistically enhance antitumor efficacy and have lowest AEs in EGFRvIII-positive rGBM. Std. treatment, standard treatment; Beva, bevacizumab; AFA, afatinib; DM, Depatux-M; TMZ, temozolomide; Rind, rindopepimut; LOM, lomustine; rGBM, recurrent glioblastoma; VEGF, vascular endothelial growth factor; TCR, T cell receptor.

DISCUSSION

This study offered a promising avenue for the treatment of rGBM in patients with EGFRvIII mutations (Figure 6). In our Bayesian NMA, we found that Rind + Beva ranked first in terms of OS improvement, followed by the regimens of DM + TMZ, DM, Std. treatment, and Beva. For PFS benefit, Rind + Beva also appears to be the most effective option, with DM + TMZ, Beva, AFA + TMZ, Std. treatment, AFA, and DM showing lower efficacy. Regarding safety, our analysis of grade $\geq$3 AEs revealed that rGBM patients commonly experienced lymphopenia, thrombocytopenia, eye disorders, neurological complications, and rash. Specifically, the DM + TMZ and DM regimens were associated with an increased incidence of eye and neurological AEs, while DM + TMZ was linked to a higher rate of lymphopenia. Additionally, AMG 595 correlated with elevated thrombocytopenia, and dacomitinib was related to a greater incidence of rash. Notably, Rind + Beva consistently exhibited the lowest incidence of both all grades and grade $\geq$3 AEs across all treatment approaches. To identify the optimal treatment strategy for EGFRvIII-positive rGBM and reinforce the evidence base for rGBM management, we systematically integrated data from 24 studies—9 focusing on ndGBM patients and 15 on rGBM cases. After applying stringent inclusion criteria, data from 7 studies specifically addressing EGFRvIII-positive rGBM were incorporated into our NMA. Notably, our NMA findings are consistent with previous clinical trials, such as a double-blind randomized phase 2 trial of rindopepimut with bevacizumab for patients with relapsed EGFRvIII-expressing GBM (ReACT) and a phase 2, multicenter, prospective trial assessing the immunogenicity of an EGFRvIII-targeted peptide vaccine (ACTIVATE),[14,20] which have demonstrated the efficacy of Rind + Beva in improving OS for EGFRvIII-positive rGBM patients.

Rather than the single target treatment approaches, combination therapy remains the most promising strategy for treating EGFRvIII mutation rGBM, particularly the approaches that focus on reshaping the immunosuppressive GBM microenvironment. Treatments targeting EGFRvIII alone, including Rind, have shown limited efficacy due to the inability to eliminate GBM cells with low or absent EGFRvIII expression. Preclinical studies indicate that Rind can induce robust antibody responses in various animal models.[27] In mice, the monoclonal antibody Y10, which exhibits EGFRvIII specificity, has been shown to inhibit DNA synthesis, impair cellular proliferation, and mediate antibody-dependent cellular cytotoxicity.[28] Moreover, studies have demonstrated that the EGFRvIII expression in GBM cells was dynamically regulated. Tumor cells can reversibly upregulate or suppress EGFRvIII expression, leading to target oncogene expression shifts that allow tumor cells to evade targeted therapy.[29] EGFRvIII-positive GBM tumors exhibit substantial molecular heterogeneity that can critically influence therapeutic response. In EGFR-amplified tumors, approximately 50%–60%

also express the EGFRvIII variant.[30] Notably, Lv et al. demonstrated that among patients with EGFR amplification, those lacking EGFRvIII expression experienced significantly longer PFS and OS following cetuximab therapy.[31] Likewise, Mellinghoff et al. reported that co-expression of EGFRvIII and PTEN conferred heightened sensitivity to EGFR tyrosine kinase inhibitors (e.g., gefitinib or erlotinib), whereas PTEN loss was associated with secondary resistance.[32] Mechanistic work by Akhavan et al. revealed that EGFRvIII signaling actively suppresses PDGFRβ transcription via mTORC1- and ERK- dependent pathways, and compensatory de-repression of PDGFRβ then promotes acquired resistance to EGFR TKIs.[33] Additionally, Yeo et al. found that EGFRvIII-driven oncogenesis requires synergistic PDGFRA signaling, such that co-expression of EGFRvIII and PDGFRA can underlie resistance to single-agent inhibitors and imply that dual targeting of both receptors may yield superior efficacy.[34] These factors cause inherent limitations on EGFRvIII single targeted therapy, highlighting the necessity of combination strategies for EGFRvIII mutation GBM. Targeting VEGF is a promising option for combination therapy with EGFRvIII targeted treatment, as VEGF inhibition not only suppresses intratumoral angiogenesis but also reverses the immunosuppressive tumor microenvironment.[35] Bevacizumab, the first available humanized monoclonal antibody targeting VEGF, was approved by FDA in 2014. It binds to all circulating VEGF-A isoforms, effectively blocking activation of VEGF signaling pathways. Early *in vivo* experiments have demonstrated that bevacizumab could induce regression of new blood vessel formation in glioma, suppress tumor growth, and prolong survival of glioma bearing mice.[36] Additionally, numerous studies have shown that bevacizumab can reshape the immunosuppressive microenvironment by promoting dendritic cells (DCs) differentiation and improving the infiltration of $CD8^+$ T cells.[37,38] The ability of Beva to promote immune cell infiltration and enhance immune function makes it a promising candidate for combination therapies with immune-modulating agents, such as PD-1/PD-L1 or CTLA-4 inhibitors. Therefore, for patients with EGFRvIII mutation, Rind + Beva may be the most effective treatment approach. Rind specifically targets and suppresses EGFRvIII expression for inhibiting its oncogenic effects, while Beva modulates the immunosuppressive tumor microenvironment for enhancing immune system activation against GBM cells.

The clinical trials have further confirmed the efficacy of Rind + Beva, supporting its potential as a first-line therapeutic option for rGBM patients with EGFRvIII mutation. The ReACT trial,[14] despite its limited sample size, validated the efficacy of this combination by showing a significant OS benefit compared to bevacizumab monotherapy (HR: 0.53, 95% CI: 0.32–0.88). Additionally, a phase 1 trial of EGFRvIII-targeted vaccine in patients with GBM (VICTORI),[39] the first DC-mediated clinical study using rindopepimut in GBM treatment, reported minimal toxicity with no autoimmune symptoms. Furthermore, the ACTIVATE trial[20] revealed that patients who exhibited a cellular immune response to rindopepimut achieved a marked OS extension (44.7 months vs. 22.8 months). However, follow-up studies indicated that the most recurrent GBM tumors eventually lost EGFRvIII expression. Moreover, the phase 3 study of rindopepimut/GM-CSF in patients with newly diagnosed EGFRvIII-expressing GBM (ACT IV)[15] demonstrated that Rind + Std. treatment failed to provide a survival advantage in newly diagnosed EGFRvIII-positive GBM (20.1 months vs. 20.0 months), suggesting the necessity of combination targeted therapies incorporating Rind to fully harness the potential of immunotherapy in GBM. Bevacizumab has been shown in two multicenter randomized controlled trials to moderately improve PFS, though not OS, in GBM patients.[40,41] In other malignancies, such as colorectal and non-small cell lung cancer, bevacizumab-containing regimens have exhibited greater efficacy when used in combination therapy.[42]

Clinical options for treating recurrent glioblastoma remain limited in efficacy at present. By integrating recommendations from the National Comprehensive Cancer Network (NCCN),[43] the European Association of Neuro-Oncology (EANO),[44] the Chinese Glioma Cooperative Group (CGCG),[45] and The Korean Society for Neuro-Oncology (KSNO) guidelines[46] (Table S7), we observed that systemic therapies are most commonly employed in recurrent GBM. Compared to other modalities, systemic treatments place fewer demands on patients—requiring only a reasonably good performance status. Experts recommend conducting molecular profiling of tumors to develop targeted systemic therapies on top of standard regimens, thereby offering renewed hope for patients with recurrent glioblastoma.[43] This study provides level-based evidence for targeted treatment strategies in recurrent GBM. Specifically, the dual-target approach of rindopepimut + bevacizumab shows promise for EGFRvIII-positive rGBM, although conclusions are constrained by the small sample sizes of the original RCTs and imbalances in prognostic factors between treatment arms. Large-scale, multicenter, double-blind RCTs will be necessary to validate these findings. However, salvage medical therapy is not the only option. Reoperation, reirradiation, stereotactic radiosurgery (SRS), tumor-treating fields (TTFs), experimental therapies, and combination regimens are frequently considered. Evidence suggests that responses may vary across molecular subgroups. The EGFRvIII-positive tumors may exhibit unique response patterns to SRS[47] or reoperation.[48]

Currently, research directly comparing pharmacologic treatments for EGFRvIII-positive GBM remains limited. Notably, only two studies[49,50] have investigated the prognostic impact of the EGFRvIII mutation, both reporting negative results. Consequently, there is insufficient evidence from evidence-based medicine to definitively guide the treatment strategies for GBM patients with this mutation. Our study underscores the potential of Rind + Beva, addressing a crucial gap in comparative efficacy research. Moreover, our comprehensive analysis offers clear, statistically robust rankings and quantitative data, providing valuable guidance for clinical decision-making. To our knowledge, this NMA is the most comprehensive and compelling comparison of the efficacy and safety of treatments for EGFRvIII-positive GBM patients to date. To ensure the strength of our evidence, we included only RCTs for primary outcomes OS and PFS, and the NMA approach helped to overcome the limitations of direct head-to-head comparisons.

Our Bayesian NMA found that the 95% CIs for all pairwise comparisons crossed the line of no effect (1.0), indicating that we cannot definitively conclude that Rind + Beva confers the best efficacy in EGFRvIII-positive rGBM. Nevertheless, this

combination remains the most promising candidate for several reasons: (1) it is the only regimen in current EGFRvIII-positive rGBM trials to demonstrate an OS benefit, despite limitations such as small sample sizes and imbalanced prognostic factors between treatment arms.[14] (2) In our forest plot comparisons, all four alternative regimens trended toward inferior OS and PFS effect estimates compared with Rind + Beva (Figures S9E and S10G). (3) Rind + Beva achieved the highest average rank, the greatest probability of being the top treatment, and the highest SUCRA score for OS, PFS, and ORR. Although ranking alone does not constitute definitive evidence of efficacy, it highlights Rind + Beva's potential as an optimal therapeutic option. (4) NCCN guidelines recommend molecular profiling in rGBM to guide targeted systemic therapies, aiming to optimize for rGBM patients. Rindopepimut is the only cancer vaccine targeting EGFRvIII among current clinical trials, and bevacizumab, an anti-VEGF antibody, is included in NCCN and KSNO guidelines for systemic treatment of rGBM (Table S7).[43,46] Moreover, combining targeted agents shows greater promise than single-agent approaches by enhancing efficacy and overcoming resistance.[51] Thus, the dual-target strategy of Rind + Beva is worthy of further validation in large, multicenter, double-blind RCTs.

The potential mechanisms underlying the synergy between rindopepimut and bevacizumab in combating EGFRvIII-positive tumors are 3-fold: (1) enhanced immune response: rindopepimut elicits EGFRvIII-specific T cell immunity capable of recognizing and killing EGFRvIII-expressing tumor cells. Concurrently, bevacizumab's VEGF blockade alleviates immunosuppression within the tumor microenvironment, permitting more robust T cell function[52]; (2) improved tumor penetration: By normalizing abnormal tumor vasculature and reducing interstitial pressure, bevacizumab decreases vascular permeability barriers, thereby facilitating rindopepimut's access to tumor sites and amplifying its immune-priming effects[53]; (3) dual-pronged tumor suppression: While rindopepimut targets a tumor-specific antigen, bevacizumab inhibits neovascularization. Together, they attack the tumor on two complementary fronts—direct immune-mediated cytotoxicity and disruption of tumor blood supply (Figure 7). In the future, the combined use of molecular-targeted vaccines and anti-VEGF monoclonal antibodies holds significant promise for precision treatment, potentially playing a pivotal role in further improving patient outcomes. We recommend that clinicians closely monitor future validation studies of this combination therapy and continuously assess the balance between efficacy and the risks of AEs, with the aim of optimizing the best individualized treatment outcomes.

In conclusion, our Bayesian NMA provides robust evidence that Rind + Beva offers promising clinical advantages for patients with EGFRvIII-positive rGBM. Specifically, Rind + Beva is associated with superior improvements in OS, PFS and ORR compared to alternative therapeutic regimens. Although DM + TMZ also demonstrates efficacy in extending OS and PFS, its safety profile is notably compromised by severe ocular and hematologic AEs. In contrast, Rind + Beva present a more favorable safety profile, with only moderate gastrointestinal discomfort and headache reported. Based on the head-to-head comparisons in the original clinical studies demonstrating an OS advantage for Rind + Beva, this study provides further evidence-based comparisons between Rind + Beva and other treatments for EGFRvIII-positive rGBM. It identifies the most likely optimal treatment strategy for these patients and offers valuable baseline evidence for a more comprehensive approach to GBM management. Nonetheless, prior to its widespread clinical adoption, additional validation through large-scale, multicenter trials is essential to confirm these preliminary findings and to comprehensively assess both the efficacy and safety of Rind + Beva.

Limitations of the study

Firstly, due to the limited number of RCTs in EGFRvIII-positive rGBM, we utilized a Bayesian method that does not rely on large-sample approximations, which strengthens the robustness of our findings. Secondly, because of the scarcity of detailed data on bevacizumab in this specific patient population, we supplemented our analysis with aggregated data from Cochrane reviews on the broader rGBM population and applied relatively broad prior distributions in our Bayesian NMA. Similarly, due to the lack of comprehensive AE data for EGFRvIII-positive rGBM patients, our safety analysis was based on the overall GBM AE profile. This limitation weakens the argument about the safety profile of Rind + Beva in the target population. We propose that future trials of EGFRvIII-targeted therapies incorporate the following measures: (1) enroll and report AEs separately for EGFRvIII-positive versus EGFRvIII-negative cohorts; (2) include EGFRvIII status in case report forms so that safety analyses can be stratified at the patient level; (3) conduct post-hoc analyses of pharmacokinetics and pharmacodynamics stratified by EGFRvIII expression to identify any unexpected safety signals; (4) stratify AEs by EGFRvIII status across all arms (e.g., immunotherapies, ADCs, VEGF inhibitors) to detect EGFRvIII-specific safety signals. Moreover, because the majority of studies included in this analysis were published prior to 2021, it is possible that some patients whose tumors were histopathologically diagnosed as glioblastoma actually harbored IDH mutations. Under the 2021 WHO classification, such tumors are now categorized as "Astrocytoma, IDH-mutant". Additionally, several studies may lack comprehensive molecular profiling of glioblastoma specimens, resulting in incomplete classification under the current guidelines. Finally, due to the lack of comprehensive data for newly diagnosed EGFRvIII-positive GBM, our systematic discussion is focused on recurrent cases. Despite these limitations, emerging studies in newly diagnosed patients have shown promising efficacy. For example, a phase 3 trial by Lassman et al.[11] demonstrated that DM significantly prolonged PFS (HR = 0.72, 95% CI: 0.56–0.93), and an IB trial by Compter et al.[17] reported that chloroquine combined with radiotherapy and TMZ extended median survival (20 months vs. 11.5 months).

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Yi Fu (fuyi@ccmu.edu.cn).

Materials availability

The study did not generate any new materials.

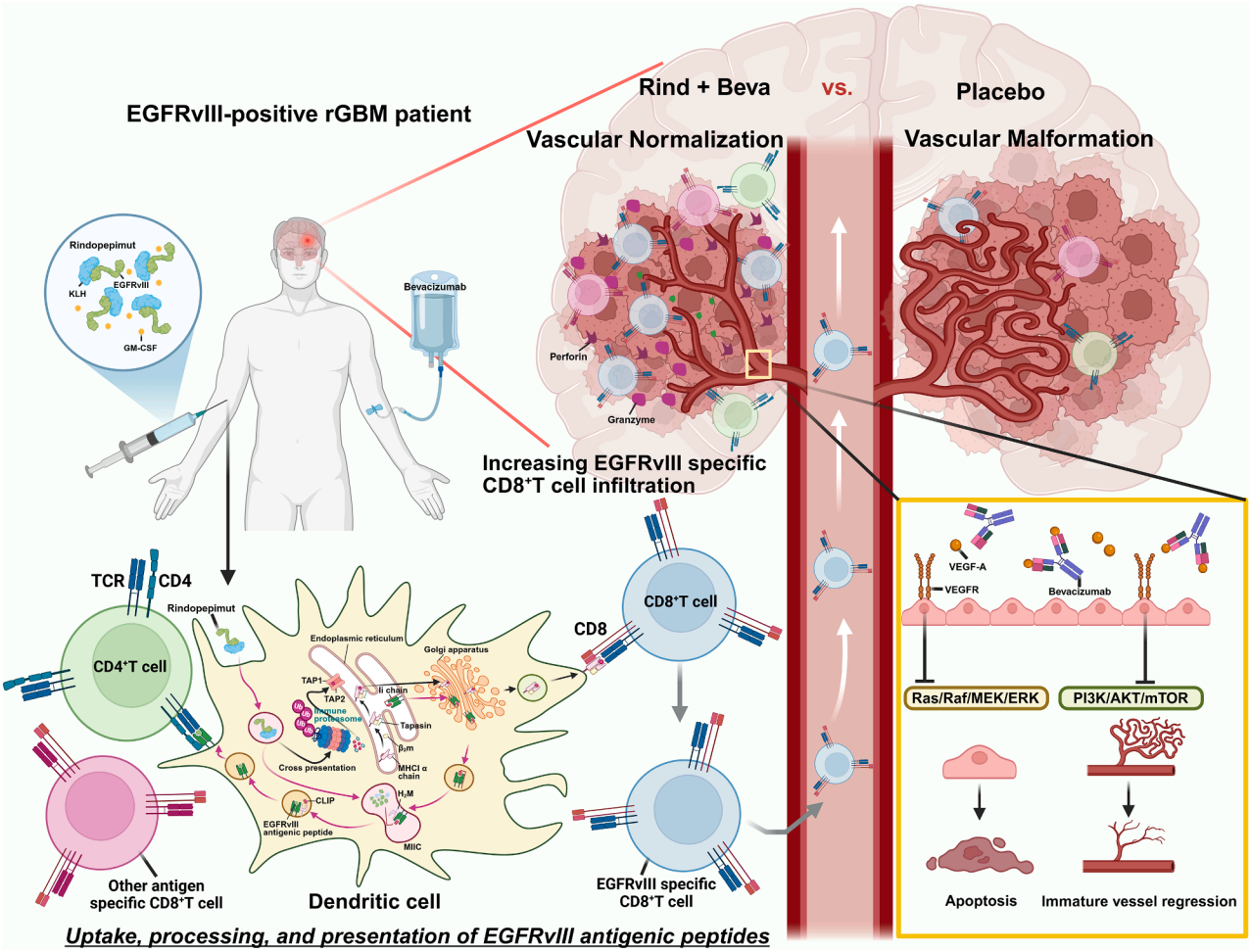

Figure 7. Potential mechanisms between VEGF blockade and immune priming

Tumor vaccines activate dendritic cells, which uptake, process, and present target antigenic peptides via MHC class I molecules through cross-presentation to naive $CD8^+$ T cells. This leads to the expansion and differentiation of antigen-specific cytotoxic $CD8^+$ T cells, which are recruited to EGFRvIII-positive glioma sites and mediate tumor cell killing through the perforin/granzyme pathway. VEGF blockade contributes by normalizing tumor vasculature and improving vascular permeability, thereby enhancing the infiltration and anti-tumor efficacy of antigen-specific $CD8^+$ T cells within the tumor microenvironment. rGBM, recurrent glioblastoma; Rind, rindopepimut; Beva, bevacizumab; VEGF, vascular endothelial growth factor; KLH, keyhole limpet hemocyanin; TAP, transporter associated with antigen processing; CLIP, class II-associated invariant chain peptide; MIIC, MHC class II compartment; H2M, histocompatibility 2; β2m, beta-2-microglobulin.

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This study did not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

J.Q.: conceptualization, methodology, software, validation, formal analysis, investigation, writing – original draft, writing review and editing, visualization,

and project administration; F.Z.: conceptualization, methodology, software, validation, formal analysis, investigation, visualization, and project administration; R.Q.: methodology, validation, formal analysis, and visualization; H.Y.: methodology, validation, formal analysis, and visualization; W.S.: methodology, validation, formal analysis, and visualization; R.W.: conceptualization, methodology, validation, formal analysis, and supervision; Y.F.: conceptualization, methodology, validation, formal analysis, writing – review and editing, and supervision. All authors designed and conducted this review.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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REFERENCES

1. Price, M., Ballard, C., Benedetti, J., Neff, C., Cioffi, G., Waite, K.A., Kruchko, C., Barnholtz-Sloan, J.S., and Ostrom, Q.T. (2024). CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2017-2021. Neuro Oncol. *26*, vi1–vi85. https://doi.org/10.1093/neuonc/noae145.
2. DeCordova, S., Shastri, A., Tsolaki, A.G., Yasmin, H., Klein, L., Singh, S.K., and Kishore, U. (2020). Molecular Heterogeneity and Immunosuppressive Microenvironment in Glioblastoma. Front. Immunol. *11*, 1402. https://doi.org/10.3389/fimmu.2020.01402.
3. Del Vecchio, C.A., Giacomini, C.P., Vogel, H., Jensen, K.C., Florio, T., Merlo, A., Pollack, J.R., and Wong, A.J. (2013). EGFRvIII gene rearrangement is an early event in glioblastoma tumorigenesis and expression defines a hierarchy modulated by epigenetic mechanisms. Oncogene *32*, 2670–2681. https://doi.org/10.1038/onc.2012.280.
4. Moscatello, D.K., Montgomery, R.B., Sundareshan, P., McDanel, H., Wong, M.Y., and Wong, A.J. (1996). Transformational and altered signal transduction by a naturally occurring mutant EGF receptor. Oncogene *13*, 85–96.
5. Inda, M.d.M., Bonavia, R., Mukasa, A., Narita, Y., Sah, D.W.Y., Vandenberg, S., Brennan, C., Johns, T.G., Bachoo, R., Hadwiger, P., et al. (2010). Tumor heterogeneity is an active process maintained by a mutant EGFR-induced cytokine circuit in glioblastoma. Genes Dev. *24*, 1731–1745. https://doi.org/10.1101/gad.1890510.
6. Emlet, D.R., Gupta, P., Holgado-Madruga, M., Del Vecchio, C.A., Mitra, S.S., Han, S.Y., Li, G., Jensen, K.C., Vogel, H., Xu, L.W., et al. (2014). Targeting a glioblastoma cancer stem-cell population defined by EGF receptor variant III. Cancer Res. *74*, 1238–1249. https://doi.org/10.1158/0008-5472.CAN-13-1407.
7. An, Z., Aksoy, O., Zheng, T., Fan, Q.W., and Weiss, W.A. (2018). Epidermal growth factor receptor and EGFRvIII in glioblastoma: signaling pathways and targeted therapies. Oncogene *37*, 1561–1575. https://doi.org/10.1038/s41388-017-0045-7.
8. Reardon, D.A., Nabors, L.B., Mason, W.P., Perry, J.R., Shapiro, W., Kavan, P., Mathieu, D., Phuphanich, S., Cseh, A., Fu, Y., et al. (2015). Phase I/randomized phase II study of afatinib, an irreversible ErbB family blocker, with or without protracted temozolomide in adults with recurrent glioblastoma. Neuro Oncol. *17*, 430–439. https://doi.org/10.1093/neuonc/nou160.
9. Gan, H.K., Burgess, A.W., Clayton, A.H.A., and Scott, A.M. (2012). Targeting of a conformationally exposed, tumor-specific epitope of EGFR as a strategy for cancer therapy. Cancer Res. *72*, 2924–2930. https://doi.org/10.1158/0008-5472.CAN-11-3898.
10. Reilly, E.B., Phillips, A.C., Buchanan, F.G., Kingsbury, G., Zhang, Y., Meulbroek, J.A., Cole, T.B., DeVries, P.J., Falls, H.D., Beam, C., et al. (2015). Characterization of ABT-806, a Humanized Tumor-Specific Anti-EGFR Monoclonal Antibody. Mol. Cancer Ther. *14*, 1141–1151. https://doi.org/10.1158/1535-7163.MCT-14-0820.
11. Lassman, A.B., Pugh, S.L., Wang, T.J.C., Aldape, K., Gan, H.K., Preusser, M., Vogelbaum, M.A., Sulman, E.P., Won, M., Zhang, P., et al. (2023). Depatuxizumab mafodotin in EGFR-amplified newly diagnosed glioblastoma: A phase III randomized clinical trial. Neuro Oncol. *25*, 339–350. https://doi.org/10.1093/neuonc/noac173.
12. Van Den Bent, M., Eoli, M., Sepulveda, J.M., Smits, M., Walenkamp, A., Frenel, J.S., Franceschi, E., Clement, P.M., Chinot, O., De Vos, F., et al. (2020). INTELLANCE 2/EORTC 1410 randomized phase II study of Depatux-M alone and with temozolomide vs temozolomide or lomustine in recurrent EGFR amplified glioblastoma. Neuro Oncol. *22*, 684–693. https://doi.org/10.1093/neuonc/noz222.
13. Rosenthal, M., Curry, R., Reardon, D.A., Rasmussen, E., Upreti, V.V., Damore, M.A., Henary, H.A., Hill, J.S., and Cloughesy, T. (2019). Safety, tolerability, and pharmacokinetics of anti-EGFRvIII antibody-drug conjugate AMG 595 in patients with recurrent malignant glioma expressing EGFRvIII. Cancer Chemother. Pharmacol. *84*, 327–336. https://doi.org/10.1007/s00280-019-03879-2.
14. Reardon, D.A., Desjardins, A., Vredenburgh, J.J., O'Rourke, D.M., Tran, D.D., Fink, K.L., Nabors, L.B., Li, G., Bota, D.A., Lukas, R.V., et al. (2020). Rindopepimut with Bevacizumab for Patients with Relapsed EGFRvIII-Expressing Glioblastoma (ReACT): Results of a Double-Blind Randomized Phase II Trial. Clin. Cancer Res. *26*, 1586–1594. https://doi.org/10.1158/1078-0432.CCR-18-1140.
15. Weller, M., Butowski, N., Tran, D.D., Recht, L.D., Lim, M., Hirte, H., Ashby, L., Mechtler, L., Goldlust, S.A., Iwamoto, F., et al. (2017). Rindopepimut with temozolomide for patients with newly diagnosed, EGFRvIII-expressing glioblastoma (ACT IV): a randomised, double-blind, international phase 3 trial. Lancet Oncol. *18*, 1373–1385. https://doi.org/10.1016/S1470-2045(17)30517-X.
16. Bagley, S.J., Binder, Z.A., Lamrani, L., Marinari, E., Desai, A.S., Nasrallah, M.P., Maloney, E., Brem, S., Lustig, R.A., Kurtz, G., et al. (2024). Repeated peripheral infusions of anti-EGFRvIII CAR T cells in combination with pembrolizumab show no efficacy in glioblastoma: a phase 1 trial. Nat. Cancer *5*, 517–531. https://doi.org/10.1038/s43018-023-00709-6.
17. Compter, I., Eekers, D.B.P., Hoeben, A., Rouschop, K.M.A., Reymen, B., Ackermans, L., Beckervordersantforth, J., Bauer, N.J.C., Anten, M.M., Wesseling, P., et al. (2021). Chloroquine combined with concurrent radiotherapy and temozolomide for newly diagnosed glioblastoma: a phase IB trial. Autophagy *17*, 2604–2612. https://doi.org/10.1080/15548627.2020.1816343.
18. van den Bent, M., Gan, H.K., Lassman, A.B., Kumthekar, P., Merrell, R., Butowski, N., Lwin, Z., Mikkelsen, T., Nabors, L.B., Papadopoulos, K.P.,

et al. (2017). Efficacy of depatuxizumab mafodotin (ABT-414) monotherapy in patients with EGFR-amplified, recurrent glioblastoma: results from a multi-center, international study. Cancer Chemother. Pharmacol. *80*, 1209–1217. https://doi.org/10.1007/s00280-017-3451-1.

19. Schuster, J., Lai, R.K., Recht, L.D., Reardon, D.A., Paleologos, N.A., Groves, M.D., Mrugala, M.M., Jensen, R., Baehring, J.M., Sloan, A., et al. (2015). A phase II, multicenter trial of rindopepimut (CDX-110) in newly diagnosed glioblastoma: the ACT III study. Neuro Oncol. *17*, 854–861. https://doi.org/10.1093/neuonc/nou348.

20. Sampson, J.H., Heimberger, A.B., Archer, G.E., Aldape, K.D., Friedman, A.H., Friedman, H.S., Gilbert, M.R., Herndon, J.E., McLendon, R.E., Mitchell, D.A., et al. (2010). Immunologic escape after prolonged progression-free survival with epidermal growth factor receptor variant III peptide vaccination in patients with newly diagnosed glioblastoma. J. Clin. Oncol. *28*, 4722–4729. https://doi.org/10.1200/JCO.2010.28.6963.

21. Prados, M.D., Chang, S.M., Butowski, N., DeBoer, R., Parvataneni, R., Carliner, H., Kabuubi, P., Ayers-Ringler, J., Rabbitt, J., Page, M., et al. (2009). Phase II study of erlotinib plus temozolomide during and after radiation therapy in patients with newly diagnosed glioblastoma multiforme or gliosarcoma. J. Clin. Oncol. *27*, 579–584. https://doi.org/10.1200/jco.2008.18.9639.

22. Brown, P.D., Krishnan, S., Sarkaria, J.N., Wu, W., Jaeckle, K.A., Uhm, J.H., Geoffroy, F.J., Arusell, R., Kitange, G., Jenkins, R.B., et al. (2008). Phase I/II trial of erlotinib and temozolomide with radiation therapy in the treatment of newly diagnosed glioblastoma multiforme: North Central Cancer Treatment Group Study N0177. J. Clin. Oncol. *26*, 5603–5609. https://doi.org/10.1200/jco.2008.18.0612.

23. Sepúlveda-Sánchez, J.M., Vaz, M.Á., Balañá, C., Gil-Gil, M., Reynés, G., Gallego, Ó., Martínez-García, M., Vicente, E., Quindós, M., Luque, R., et al. (2017). Phase II trial of dacomitinib, a pan-human EGFR tyrosine kinase inhibitor, in recurrent glioblastoma patients with EGFR amplification. Neuro Oncol. *19*, 1522–1531. https://doi.org/10.1093/neuonc/nox105.

24. Taal, W., Oosterkamp, H.M., Walenkamp, A.M.E., Dubbink, H.J., Beerepoot, L.V., Hanse, M.C.J., Buter, J., Honkoop, A.H., Boerman, D., de Vos, F.Y.F., et al. (2014). Single-agent bevacizumab or lomustine versus a combination of bevacizumab plus lomustine in patients with recurrent glioblastoma (BELOB trial): a randomised controlled phase 2 trial. Lancet Oncol. *15*, 943–953. https://doi.org/10.1016/S1470-2045(14)70314-6.

25. ClinicalTrials.gov (2021). Identifier NCT02844439, Study of Tesevatinib Monotherapy in Patients With Recurrent Glioblastoma (Bethesda (MD): National Library of Medicine (US)). https://clinicaltrials.gov/study/NCT02844439.

26. Zhao, B., Wu, J., Xia, Y., Li, H., Wang, Y., Qu, T., Xing, H., Wang, Y., and Ma, W. (2022). Comparative efficacy and safety of therapeutics for elderly glioblastoma patients: A Bayesian network analysis. Pharmacol. Res. *182*, 106316. https://doi.org/10.1016/j.phrs.2022.106316.

27. Wikstrand, C.J., Stanley, S.D., Humphrey, P.A., Pegram, C.N., Archer, G. E., Kurpad, S., Shibuya, M., and Bigner, D.D. (1993). Investigation of a synthetic peptide as immunogen for a variant epidermal growth factor receptor associated with gliomas. J. Neuroimmunol. *46*, 165–173. https://doi.org/10.1016/0165-5728(93)90246-u.

28. Wikstrand, C.J., Hale, L.P., Batra, S.K., Hill, M.L., Humphrey, P.A., Kurpad, S.N., McLendon, R.E., Moscatello, D., Pegram, C.N., Reist, C.J., et al. (1995). Monoclonal antibodies against EGFRvIII are tumor specific and react with breast and lung carcinomas and malignant gliomas. Cancer Res. *55*, 3140–3148.

29. Nathanson, D.A., Gini, B., Mottahedeh, J., Visnyei, K., Koga, T., Gomez, G., Eskin, A., Hwang, K., Wang, J., Masui, K., et al. (2014). Targeted therapy resistance mediated by dynamic regulation of extrachromosomal mutant EGFR DNA. Science *343*, 72–76. https://doi.org/10.1126/science.1241328.

30. Felsberg, J., Hentschel, B., Kaulich, K., Gramatzki, D., Zacher, A., Malzkorn, B., Kamp, M., Sabel, M., Simon, M., Westphal, M., et al. (2017). Epidermal Growth Factor Receptor Variant III (EGFRvIII) Positivity in EGFR-Amplified Glioblastomas: Prognostic Role and Comparison between Primary and Recurrent Tumors. Clin. Cancer Res. *23*, 6846–6855. https://doi.org/10.1158/1078-0432.CCR-17-0890.

31. Lv, S., Teugels, E., Sadones, J., De Brakeleer, S., Duerinck, J., Du Four, S., Michotte, A., De Grève, J., and Neyns, B. (2012). Correlation of EGFR, IDH1 and PTEN status with the outcome of patients with recurrent glioblastoma treated in a phase II clinical trial with the EGFR-blocking monoclonal antibody cetuximab. Int. J. Oncol. *41*, 1029–1035. https://doi.org/10.3892/ijo.2012.1539.

32. Mellinghoff, I.K., Wang, M.Y., Vivanco, I., Haas-Kogan, D.A., Zhu, S., Dia, E.Q., Lu, K.V., Yoshimoto, K., Huang, J.H.Y., Chute, D.J., et al. (2005). Molecular determinants of the response of glioblastomas to EGFR kinase inhibitors. N. Engl. J. Med. *353*, 2012–2024. https://doi.org/10.1056/NEJMoa051918.

33. Akhavan, D., Pourzia, A.L., Nourian, A.A., Williams, K.J., Nathanson, D., Babic, I., Villa, G.R., Tanaka, K., Nael, A., Yang, H., et al. (2013). De-repression of PDGFRbeta transcription promotes acquired resistance to EGFR tyrosine kinase inhibitors in glioblastoma patients. Cancer Discov. *3*, 534–547. https://doi.org/10.1158/2159-8290.CD-12-0502.

34. Yeo, A.T., Jun, H.J., Appleman, V.A., Zhang, P., Varma, H., Sarkaria, J.N., and Charest, A. (2021). EGFRvIII tumorigenicity requires PDGFRA cosignaling and reveals therapeutic vulnerabilities in glioblastoma. Oncogene *40*, 2682–2696. https://doi.org/10.1038/s41388-021-01721-9.

35. Cheng, S.Y., Huang, H.J., Nagane, M., Ji, X.D., Wang, D., Shih, C.C., Arap, W., Huang, C.M., and Cavenee, W.K. (1996). Suppression of glioblastoma angiogenicity and tumorigenicity by inhibition of endogenous expression of vascular endothelial growth factor. Proc. Natl. Acad. Sci. USA *93*, 8502–8507. https://doi.org/10.1073/pnas.93.16.8502.

36. von Baumgarten, L., Brucker, D., Tirniceru, A., Kienast, Y., Grau, S., Burgold, S., Herms, J., and Winkler, F. (2011). Bevacizumab has differential and dose-dependent effects on glioma blood vessels and tumor cells. Clin. Cancer Res. *17*, 6192–6205. https://doi.org/10.1158/1078-0432.CCR-10-1868.

37. Kim, M.M., Umemura, Y., and Leung, D. (2018). Bevacizumab and Glioblastoma: Past, Present, and Future Directions. Cancer J. *24*, 180–186. https://doi.org/10.1097/PPO.0000000000000326.

38. Boucher, Y., Kumar, A.S., Posada, J.M., Gjini, E., Pfaff, K., Lipschitz, M., Lako, A., Duda, D.G., Rodig, S.J., Hodi, F.S., and Jain, R.K. (2021). Bevacizumab improves tumor infiltration of mature dendritic cells and effector T-cells in triple-negative breast cancer patients. npj Precis. Oncol. *5*, 62. https://doi.org/10.1038/s41698-021-00197-w.

39. Sampson, J.H., Archer, G.E., Mitchell, D.A., Heimberger, A.B., Herndon, J. E., 2nd, Lally-Goss, D., McGehee-Norman, S., Paolino, A., Reardon, D.A., Friedman, A.H., et al. (2009). An epidermal growth factor receptor variant III-targeted vaccine is safe and immunogenic in patients with glioblastoma multiforme. Mol. Cancer Ther. *8*, 2773–2779. https://doi.org/10.1158/1535-7163.MCT-09-0124.

40. Kreisl, T.N., Kim, L., Moore, K., Duic, P., Royce, C., Stroud, I., Garren, N., Mackey, M., Butman, J.A., Camphausen, K., et al. (2009). Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. J. Clin. Oncol. *27*, 740–745. https://doi.org/10.1200/JCO.2008.16.3055.

41. Hurwitz, H.I., Fehrenbacher, L., Hainsworth, J.D., Heim, W., Berlin, J., Holmgren, E., Hambleton, J., Novotny, W.F., and Kabbinavar, F. (2005). Bevacizumab in combination with fluorouracil and leucovorin: an active regimen for first-line metastatic colorectal cancer. J. Clin. Oncol. *23*, 3502–3508. https://doi.org/10.1200/JCO.2005.10.017.

42. Kerr, C. (2005). Bevacizumab and chemotherapy improves survival in NSCLC. Lancet Oncol. *6*, 266. https://doi.org/10.1016/s1470-2045(05)70155-8.

43. Network National Comprehensive Cancer. Central Nervous System Cancers (Version 5.2024). NCCN Clinical Practice Guidelines in Oncology; 2024. https://www.nccn.org/professionals/physician_gls/pdf/cns.pdf.

44. Weller, M., van den Bent, M., Preusser, M., Le Rhun, E., Tonn, J.C., Minniti, G., Bendszus, M., Balana, C., Chinot, O., Dirven, L., et al. (2021). EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood. Nat. Rev. Clin. Oncol. *18*, 170–186. https://doi.org/10.1038/s41571-020-00447-z.

45. Jiang, T., Nam, D.H., Ram, Z., Poon, W.S., Wang, J., Boldbaatar, D., Mao, Y., Ma, W., Mao, Q., You, Y., et al. (2021). Clinical practice guidelines for the management of adult diffuse gliomas. Cancer Lett. *499*, 60–72. https://doi.org/10.1016/j.canlet.2020.10.050.

46. Kim, Y.Z., Kim, C.Y., Lim, J., Sung, K.S., Lee, J., Oh, H.J., Kang, S.G., Kang, S.H., Kong, D.S., Kim, S.H., et al. (2019). The Korean Society for Neuro-Oncology (KSNO) Guideline for Glioblastomas: Version 2018.01. Brain Tumor Res. Treat. *7*, 1–9. https://doi.org/10.14791/btrt.2019.7.e25.

47. Dono, A., Amsbaugh, M., Martir, M., Smilie, R.H., Riascos, R.F., Zhu, J.J., Hsu, S., Kim, D.H., Tandon, N., Ballester, L.Y., et al. (2021). Genomic alterations predictive of response to radiosurgery in recurrent IDH-WT glioblastoma. J. Neuro Oncol. *152*, 153–162. https://doi.org/10.1007/s11060-020-03689-0.

48. Dono, A., Zhu, P., Takayasu, T., Arevalo, O., Riascos, R., Tandon, N., Ballester, L.Y., and Esquenazi, Y. (2024). Extent of Resection Thresholds in Molecular Subgroups of Newly Diagnosed Isocitrate Dehydrogenase-Wildtype Glioblastoma. Neurosurgery *95*, 932–940. https://doi.org/10.1227/neu.0000000000002964.

49. Hoogstrate, Y., Ghisai, S.A., de Wit, M., de Heer, I., Draaisma, K., van Riet, J., van de Werken, H.J.G., Bours, V., Buter, J., Vanden Bempt, I., et al. (2022). The EGFRvIII transcriptome in glioblastoma: A meta-omics analysis. Neuro Oncol. *24*, 429–441. https://doi.org/10.1093/neuonc/noab231.

50. Chen, J.R., Xu, H.Z., Yao, Y., and Qin, Z.Y. (2015). Prognostic value of epidermal growth factor receptor amplification and EGFRvIII in glioblastoma: meta-analysis. Acta Neurol. Scand. *132*, 310–322. https://doi.org/10.1111/ane.12401.

51. Shen, Y., Thng, D.K.H., Wong, A.L.A., and Toh, T.B. (2024). Mechanistic insights and the clinical prospects of targeted therapies for glioblastoma: a comprehensive review. Exp. Hematol. Oncol. *13*, 40. https://doi.org/10.1186/s40164-024-00512-8.

52. de Almeida, P.E., Mak, J., Hernandez, G., Jesudason, R., Herault, A., Javinal, V., Borneo, J., Kim, J.M., and Walsh, K.B. (2020). Anti-VEGF Treatment Enhances CD8(+) T-cell Antitumor Activity by Amplifying Hypoxia. Cancer Immunol. Res. *8*, 806–818. https://doi.org/10.1158/2326-6066.CIR-19-0360.

53. Qian, C., Liu, C., Liu, W., Zhou, R., and Zhao, L. (2023). Targeting vascular normalization: a promising strategy to improve immune-vascular crosstalk in cancer immunotherapy. Front. Immunol. *14*, 1291530. https://doi.org/10.3389/fimmu.2023.1291530.

54. Hutton, B., Salanti, G., Caldwell, D.M., Chaimani, A., Schmid, C.H., Cameron, C., Ioannidis, J.P.A., Straus, S., Thorlund, K., Jansen, J.P., et al. (2015). The PRISMA extension statement for reporting of systematic reviews incorporating network meta-analyses of health care interventions: checklist and explanations. Ann. Intern. Med. *162*, 777–784. https://doi.org/10.7326/M14-2385.

55. Board WHO Classification of Tumours Editorial (2021). World Health Organization Classification of Tumours of the Central Nervous System (Lyon: International Agency for Research on Cancer), pp. 5–34.

56. Wen, P.Y., van den Bent, M., Youssef, G., Cloughesy, T.F., Ellingson, B.M., Weller, M., Galanis, E., Barboriak, D.P., de Groot, J., Gilbert, M.R., et al. (2023). RANO 2.0: Update to the Response Assessment in Neuro-Oncology Criteria for High- and Low-Grade Gliomas in Adults. J. Clin. Oncol. *41*, 5187–5199. https://doi.org/10.1200/JCO.23.01059.

57. Wen, P.Y., Macdonald, D.R., Reardon, D.A., Cloughesy, T.F., Sorensen, A.G., Galanis, E., Degroot, J., Wick, W., Gilbert, M.R., Lassman, A.B., et al. (2010). Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. J. Clin. Oncol. *28*, 1963–1972. https://doi.org/10.1200/JCO.2009.26.3541.

58. Macdonald, D.R., Cascino, T.L., Schold, S.C., Jr., and Cairncross, J.G. (1990). Response criteria for phase II studies of supratentorial malignant glioma. J. Clin. Oncol. *8*, 1277–1280. https://doi.org/10.1200/JCO.1990.8.7.1277.

59. McBain, C., Lawrie, T.A., Rogozińska, E., Kernohan, A., Robinson, T., and Jefferies, S. (2021). Treatment options for progression or recurrence of glioblastoma: a network meta-analysis. Cochrane Database Syst. Rev. *5*, CD013579. https://doi.org/10.1002/14651858.CD013579.pub2.

60. Higgins, J.P.T., Altman, D.G., Gøtzsche, P.C., Jüni, P., Moher, D., Oxman, A.D., Savovic, J., Schulz, K.F., Weeks, L., Sterne, J.A.C., et al. (2011). The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ *343*, d5928. https://doi.org/10.1136/bmj.d5928.

61. Guyatt, G.H., Oxman, A.D., Vist, G.E., Kunz, R., Falck-Ytter, Y., Alonso-Coello, P., and Schünemann, H.J.; GRADE Working Group (2008). GRADE: an emerging consensus on rating quality of evidence and strength of recommendations. BMJ *336*, 924–926. https://doi.org/10.1136/bmj.39489.470347.AD.

62. Musazadeh, V., Assadian, K., Rajabi, F., Faghfouri, A.H., Soleymani, Y., Kavyani, Z., and Najafiyan, B. (2024). The effect of synbiotics on liver enzymes, obesity indices, blood pressure, lipid profile, and inflammation in patients with non-alcoholic fatty liver: A systematic review and meta-analysis of randomized controlled trials. Pharmacol. Res. *208*, 107398. https://doi.org/10.1016/j.phrs.2024.107398.

63. Tierney, J.F., Stewart, L.A., Ghersi, D., Burdett, S., and Sydes, M.R. (2007). Practical methods for incorporating summary time-to-event data into meta-analysis. Trials *8*, 16. https://doi.org/10.1186/1745-6215-8-16.

64. Liu, L., Bai, H., Wang, C., Seery, S., Wang, Z., Duan, J., Li, S., Xue, P., Wang, G., Sun, Y., et al. (2021). Efficacy and Safety of First-Line Immunotherapy Combinations for Advanced NSCLC: A Systematic Review and Network Meta-Analysis. J. Thorac. Oncol. *16*, 1099–1117. https://doi.org/10.1016/j.jtho.2021.03.016.

65. Caldwell, D.M., Ades, A.E., and Higgins, J.P.T. (2005). Simultaneous comparison of multiple treatments: combining direct and indirect evidence. BMJ *331*, 897–900. https://doi.org/10.1136/bmj.331.7521.897.

66. Mavridis, D., and Salanti, G. (2013). A practical introduction to multivariate meta-analysis. Stat. Methods Med. Res. *22*, 133–158. https://doi.org/10.1177/0962280211432219.

67. Dias, S., Sutton, A.J., Ades, A.E., and Welton, N.J. (2013). Evidence synthesis for decision making 2: a generalized linear modeling framework for pairwise and network meta-analysis of randomized controlled trials. Med. Decis. Mak. *33*, 607–617. https://doi.org/10.1177/0272989X12458724.

68. Spiegelhalter, D.J., Best, N.G., Carlin, B.P., and van der Linde, A. (2002). Bayesian measures of model complexity and fit. J. Roy. Stat. Soc. B Stat. Methodol. *64*, 583–639. https://doi.org/10.1111/1467-9868.00353.

69. Salanti, G., Ades, A.E., and Ioannidis, J.P.A. (2011). Graphical methods and numerical summaries for presenting results from multiple-treatment meta-analysis: an overview and tutorial. J. Clin. Epidemiol. *64*, 163–171. https://doi.org/10.1016/j.jclinepi.2010.03.016.

70. Wick, W., Gorlia, T., Bendszus, M., Taphoorn, M., Sahm, F., Harting, I., Brandes, A.A., Taal, W., Domont, J., Idbaih, A., et al. (2017). Lomustine and Bevacizumab in Progressive Glioblastoma. N. Engl. J. Med. *377*, 1954–1963. https://doi.org/10.1056/NEJMoa1707358.

71. Brandes, A.A., Gil-Gil, M., Saran, F., Carpentier, A.F., Nowak, A.K., Mason, W., Zagonel, V., Dubois, F., Finocchiaro, G., Fountzilas, G., et al. (2019). A Randomized Phase II Trial (TAMIGA) Evaluating the Efficacy and Safety of Continuous Bevacizumab Through Multiple Lines of Treatment for Recurrent Glioblastoma. Oncologist *24*, 521–528. https://doi.org/10.1634/theoncologist.2018-0290.

72. Egger, M., Davey Smith, G., Schneider, M., and Minder, C. (1997). Bias in meta-analysis detected by a simple, graphical test. BMJ *315*, 629–634.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	Source	IDENTIFIER
Deposited data		
International prospective register of systematic reviews	https://www.crd.york.ac.uk/PROSPERO/view/CRD42024623289	PROSPERO
PubMed	https://pubmed.ncbi.nlm.nih.gov/	N/A
Web of Science	https://www.webofscience.com/wos/	N/A
Embase	https://www.embase.com/	N/A
Cochrane Library	https://www.cochranelibrary.com/	N/A
ClinicalTrials.gov	https://clinicaltrials.gov/	N/A
Software and algorithms		
EndNote	https://support.clarivate.com/Endnote/s/article/Download-EndNote?language=zh_CN	Endnote 21
RevMan	https://www.cochrane.org/learn/courses-and-resources/software	Version 5.4
R	https://www.r-project.org/	Version 4.4.1
JAGS	https://mcmc-jags.sourceforge.io/	Version 4.3.2
WinBUGS	https://winbugs.software.informer.com/	Version 1.4.3

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Our study does not use experimental models typical in the life sciences. A total of 716 patients were enrolled across the included studies, all of whom were histologically confirmed to have glioblastoma and were generally consistent with the 2021 WHO Classification of Tumors of the Central Nervous System. All patients received one of the following ten treatment regimens: standard treatment, DM, DM + TMZ, AFA, AFA + TMZ, Beva, Rind + Beva, AMG 595, Tesevatinib, or Dacomitinib. Detailed characteristics of all included studies are presented in Tables 1, S5, and S6. Ethical approval is not required for this study because the original clinical trials had previously received authorization from the ethics and institutional review board.

METHOD DETAILS

Study registration and reporting standards

This study was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension statement for network meta-analyses (Table S1).[54] The meta-analysis protocol was registered on PROSPERO website (https://www.crd.york.ac.uk/PROSPERO) under the following registration number: CRD42022303279.

Search strategy

The search strategy is given in Table S2. We reviewed PubMed, Web of Science, EMBASE, Cochrane Library and Clinical Trials.gov for related literature from inception to Oct. 31, 2024. The following keywords were used: ''EGFR variant III'', ''EGFRvIII mutation'', ''EGFR exon 2–7 deletion'', ''Glioblastoma'', ''Astrocytomas, Grade IV'', ''Randomized controlled trials'' and ''Clinical trials as topic''. No restrictions were applied on the language. Reference lists of the retrieved studies were also manually searched.

Selection criteria

Searched articles were initially screened by two authors (Jm-Qiu and Fg-Zhu) by their titles and abstracts. The full texts of potentially included studies were reviewed by the same two authors, and any disagreements were resolved with a discussion in a panel involving other authors who are experts in oncology and evidence-based medicine (Y-Fu and Renxi-Wang).

Published and gray sources that met the following criteria were included: (1) Clinical trials enrolling patients with glioblastoma, as confirmed at least by histological analysis and classified according to the 2021 WHO Classification of Tumors of the Central Nervous System (CNS) as ''Glioblastoma, IDH wildtype''[55]; (2) Clinical trials recruiting patients with recurrent or progressive glioblastoma, as defined according to Macdonald/RANO criteria, with the minimum requirement of magnetic resonance imaging (MRI) demonstrating at least one bi-dimensionally measurable target lesion (tumor $\geq$10 mm in one diameter)[56–58]; (3) Clinical trials enrolling patients with

EGFRvIII-positive rGBM, as confirmed through histological or cytological analysis; (4) Clinical trials assessing pharmacological therapies, including but not limited to targeted therapies, immunotherapies, chemotherapies, or combination therapies; (5) Clinical trials comparing the current standard of care without a focus on molecular targets with other treatment modalities for patients with recurrent EGFRvIII-positive rGBM; (6) All phases of clinical trials reporting at least one of the following clinical outcomes; (7) OS referring to the length of time from the start of treatment until the death of the patient from any cause; (8) PFS referring to the length of time from start of treatment (or randomization) until disease progression or death, whichever occurs first; (9) ORR referring to the proportion of patients who experience a complete response (CR) or partial response (PR) to treatment, based on standardized criteria for assessing tumor response; (10) AEs, encompassing both any-grade occurrences and those of Grade $\geq$3 severity, were systematically defined and graded in accordance with the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE).

The exclusion criteria were as follows: (1) Clinical trials that enrolled mixed populations without conducting stratified or subgroup analyses specifically for EGFRvIII-positive patients; (2) Clinical trials that did not report clinically relevant outcomes essential for evaluating treatment efficacy or safety; (3) Publications classified as review articles, editorials, or case reports, as well as those not meeting the criteria for original research; (4) Studies centered on animal models or preclinical experimental data without clinical application in human subjects.

Data extraction and treatment arms

The useful information was extracted by two independent authors (R-Quan and Hy-Ye) following the prespecified protocol. The trial name, first author, publication sources, year of publication, trial phase, National Clinical Trials identification number, sample size, patients' age and sex distribution, time since diagnosis of recurrence/progression, EGFRvIII mutation status, and ECOG (Eastern Cooperative Oncology Group) or WHO (World Health Organization) performance status score were extracted from each article. The clinical outcomes extracted included HRs with corresponding 95% CIs for OS and PFS and the incidence of ORR, any-grade AEs, and grade $\geq$3 AEs.

Currently, we identified a total of 10 types of interventions in rGBM patients: standard treatment (Std. treatment/ST) (Lomustine or TMZ), DM, DM + TMZ, Afatinib (AFA), AFA + TMZ, Beva, Rind + Beva, AMG 595, Tesevatinib, Dacomitinib.

Given the limited availability of data specific to bevacizumab in EGFRvIII positive rGBM, we incorporated aggregated data from studies reporting on the overall population of recurrent glioblastoma, as referenced in the Cochrane review by McBain C et al.[59] These data were used as a replacement for EGFRvIII positive cohorts, acknowledging this as an approximation. Sensitivity analyses were planned to assess the robustness of this assumption.

Quality assessment

To assess the quality of the included studies, we utilized the modified Cochrane Collaboration's Risk of Bias (RoB) tool.[60] This tool evaluates seven domains: randomization sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective reporting, and other potential biases. Each study was categorized as having a high, low, or unclear risk of bias based on these domains. Two coauthors (Jm-Qiu and Fg-Zhu) independently assessed all included RCTs using the RoB tool. In cases of disagreement, the studies were re-evaluated, and a consensus was reached through discussion. This systematic approach ensured the reliability and accuracy of the quality assessment.

Evidence grade evaluation

We employed the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) framework to determine the quality of evidence for OS and PFS, classifying it as very low, low, moderate, or high. The GRADE system evaluates eight factors: risk of bias, imprecision, inconsistency, indirectness, publication bias, large effect size, dose–response relationship, and residual confounding that could diminish the effect size. According to this framework, the evidence was downgraded to "moderate" if one domain was rated as "serious". If two domains were rated as "serious", the evidence was further downgraded to "low", and if two or more domains were rated as "serious", it was downgraded to "very low".[61,62] This systematic evaluation ensured a rigorous assessment of the reliability and applicability of the evidence.

QUANTIFICATION AND STATISTICAL ANALYSIS

We extracted detailed OS, PFS and ORR data for all GBM patients included in the trials, as well as for those with EGFRvIII-positive GBM. HRs and 95% CIs for OS were either directly obtained from the original studies or calculated using the algorithm recommended by Tierney et al.[63] In addition, the HRs for OS reported in the TMZ or Lomustine arms of each study were aggregated to form the Std. treatment group for subsequent analysis. All grade and $\geq$3 grade AEs were reviewed and deposited in standardized tables.

Indirect comparisons of the effectiveness of various therapies for EGFRvIII-positive rGBM were performed within a Bayesian framework using Markov Chain Monte Carlo (MCMC) simulation methods. The primary analyses were conducted using WinBUGS software (version 1.4.3), part of the Bayesian Inference Using Gibbs Sampling (BUGS) project, which facilitates practical implementation of MCMC methods for applied statisticians. To validate the results, the analysis was replicated in R software (version 4.4.1) using the package gemtc (version 1.0–2) and JAGS software (version 4.3.2) with identical parameter settings. A random-effects consistency model was adopted for each outcome measure. Four independent Markov chains were initialized, with 20,000 burn-ins

followed by 100,000 sample iterations per chain, using a step-size iteration of one. The posterior distributions for treatment effects were obtained, and model convergence was evaluated using trace plots (Figures S3–S5) and the Brooks-Gelman-Rubin diagnostic (Figures S6–S8).[64] The fit of the random-effects model was assessed by the deviance information criteria (DIC).[65,66] Furthermore, the DIC difference between consistency and inconsistency models was examined to ensure coherence of the network. A large difference in DIC (>5) between the two models suggests evidence of inconsistency in the network.[67,68]

A probability ranking analysis was performed and cumulative ranking plots were generated. We employed the surface under the cumulative ranking curve (SUCRA) index to determine the relative efficacy of each treatment modality and to identify the optimal treatment option. As far as we know, the SUCRA value encapsulates all possible ranking scenarios and reflects the uncertainty associated with the treatment effects. If the SUCRA value was close to 1, it was the best without uncertainty; if the value was close to 0, it was the worst without uncertainty.[69] Thus, rankings could be determined according to the distinct SUCRA of each treatment. For the NMA process, statistical significance was established when the 95% CIs did not cover 1.

In the sensitivity analysis, we allowed the HRs for OS and PFS from the Cochrane review by McBain C et al.[59] on bevacizumab treatment to vary by ±10%. Furthermore, we merged the bevacizumab treatment node with the standard treatment node to account for potential heterogeneity.[69] The merging of the Bevacizumab and Standard treatment nodes during our sensitivity analysis was justified by multiple lines of evidence: (1) Taal et al. demonstrated no significant difference in OS between Bevacizumab monotherapy and Lomustine monotherapy, supporting clinical equivalence[24]; (2) Wick et al. found that adding Bevacizumab to Lomustine did not significantly improve OS versus Lomustine alone, further confirming equivalence with standard salvage chemotherapy[70]; (3) Brandes et al. observed no survival benefit or detriment from continued Bevacizumab across multiple lines of therapy in recurrent GBM patients[71]; (4) Cochrane review concluded there may be little or no difference in OS between Bevacizumab and Lomustine monotherapies (HR 1.22, 95% CI 0.84–1.76), and similarly for PFS (HR 0.90, 95% CI 0.58–1.38)[59]; (5) Clinical guidelines NCCN and KSNO list Bevacizumab and Lomustine as equivalent systemic therapy options for rGBM without prioritizing one over the other, indicating that Bevacizumab is considered an acceptable alternative to standard salvage chemotherapy.[43,46] Using a Bayesian random-effects model and applying MCMC methods, we fitted the posterior distributions and generated a ranking, which was then compared with the original results to further confirm the NMA findings.

To assess publication bias, we constructed funnel plots and performed Egger's test.[72] A p-value below 0.10 was interpreted as evidence of significant funnel plot asymmetry, suggestive of potential publication bias. All two-sided P-values <0.05 were considered statistically significant.