Appendix

METHODS

Study Assessments

The Central Radiology Review Committee (CRRC) performed a prospective review in patients with investigator-determined radiologic progression at first or second magnetic resonance imaging (MRI) scan, to ensure discontinuation was not prematurely decided due to pseudoprogression (defined as a non-tumor-related increase in contrast enhancement seen on MRI scan, typically occurring within the first 12 weeks following concomitant radiotherapy/temozolomide (RT/TMZ), which then stabilizes or decreases over time in the absence of treatment modification). If subsequent imaging showed progression, the time of progression was backdated. A central efficacy read was performed by two independent radiologists in parallel and adjudicated when needed; additionally, the adjudicated radiology data were reviewed alongside clinical data by an independent oncologist. Objective response rate (ORR; complete or partial response [> 50%] on two consecutive occasions ≥ 4 weeks apart) was evaluated in patients with measurable disease at baseline and based on the blinded evaluation by the CRRC using Response Assessment in Neuro-Oncology criteria [Wen et al 2010].

DNA for the assessment of O6-methylguanine-DNA methyltransferase (MGMT) (by OncoMethylome's MGMT assay) and histone status were was extracted from sections of formalin-fixed paraffin-embedded tissue blocks, which were collected at the time of central pathology review.

Safety

Adverse events (AEs) were graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events (version 4.0). AEs of special interest were based on grouping AE terms by specific Medical Dictionary for Regulatory Activities (MedDRA) baskets and standard MedDRA queries and included: hypertension grade ≥ 3; proteinuria grade ≥ 3; gastrointestinal (GI) perforation, abscesses or fistula (any grade);

wound healing complications grade ≥ 3; hemorrhage grade ≥ 3 (any grade central nervous system bleeding; grade 2 hemoptysis); arterial thromboembolic events (any grade); venous thromboembolic events grade ≥ 3; posterior reversible encephalopathy syndrome or reversible posterior leukoencephalopathy syndrome (any grade); congestive heart failure grade ≥ 3; non-GI fistula or abscess grade ≥ 2.

Statistical Analysis

The sample size of this study was based on pragmatic considerations. A total of 120 patients was considered achievable over 3 years of accrual. Assuming an exponential model with a hazard ratio (HR) of 0.65 between the treatment groups (1-year event-free survival [EFS] of 30% in the RT/TMZ group and 46% in the bevacizumab [BEV]+RT/TMZ group), the power of the log-rank test (two-sided, with alpha of 5%) for a sample size of 60 patients per group was 60%.

A pre-specified interim futility analysis was performed based on the first 60 randomized patients who were followed for 1 year; if a protocol-specified threshold of a 10% greater improvement in 1-year EFS rate in the BEV+RT/TMZ group versus the RT/TMZ group was not met, the study would be considered futile.

Pre-specified sensitivity analyses included an unstratified analysis of CRRC-assessed EFS, and CRRC-assessed EFS with censoring of the data for patients who discontinued the study for any reason prior to experiencing an EFS event. Exploratory multivariate Cox regression analyses were conducted to assess the impact of prognostic factors on EFS (age, bone age at baseline, high-grade glioma [HGG] grade, complete resection or biopsy, *MGMT* gene promotor status, histone mutation status, Karnofsky performance status, and Lansky play-performance status). As an additional exploratory, post-hoc sensitivity analysis, the heterogeneity of the effect of BEV according to stratification variables and other potential baseline prognostic factors was assessed in multivariable models (including interaction terms) and shown in a forest plot.

RESULTS

Patients

174 patients were screened for enrollment and 53 patients failed screening. The most common reasons for screen failure included failure to meet the following criteria: confirmation of local histological diagnosis by a designated central reference neuropathologist and newly diagnosed localized, supratentorial or infratentorial cerebellar or peduncular, WHO Grade III or Grade IV gliomas after central radiological review.

Patient Disposition and Protocol Deviations

Patients received study treatment at 51 sites in 14 countries (Australia, Austria, Belgium, Canada, Czech Republic, Denmark, France, Hungary, Italy, the Netherlands, Poland, Spain, Sweden, and the UK).

The most common reasons for screening failure were no confirmation of a local histologic diagnosis by a designated central reference neuropathologist (n=17) and absence of a newly diagnosed localized, supratentorial or infratentorial cerebellar or peduncular World Health Organization (WHO) grade III or IV glioma (n=9); 5% of patients had non-eligible low-grade glioma and were excluded.

There were 14 major protocol deviations (RT/TMZ, n=8; BEV+RT/TMZ, n=6), including continued study treatment after experiencing progressive disease (PD) or an AE that warranted treatment discontinuation (RT/TMZ, n=8; BEV+RT/TMZ, n=5). One patient in the BEV+RT/TMZ group underwent surgery prior to PD. There was no cross-over between treatment groups.

Sensitivity Analyses of the Primary Efficacy Endpoint

Results of sensitivity analyses performed on CRRC-assessed EFS were consistent with the primary analysis (Appendix Table A1, online only).

Health-Related Quality of Life

Mean baseline absolute scores for the Health Utility Index (HUI) questionnaire were balanced between the treatment groups (0.97 with RT/TMZ [n=40] and 0.96 with BEV+RT/TMZ [n=46]). At cycle 6 (day 1), the mean change from baseline in HUI score was −0.004 and 0.034 for the RT/TMZ (n=24) and BEV+RT/TMZ (n=35) groups, respectively (a change in mean overall HUI scores of 0.03 is considered a clinically meaningful difference) [Horsman et al 2003]. However, only four patients in the RT/TMZ group and three patients in the BEV+RT/TMZ group completed the questionnaire at follow-up.

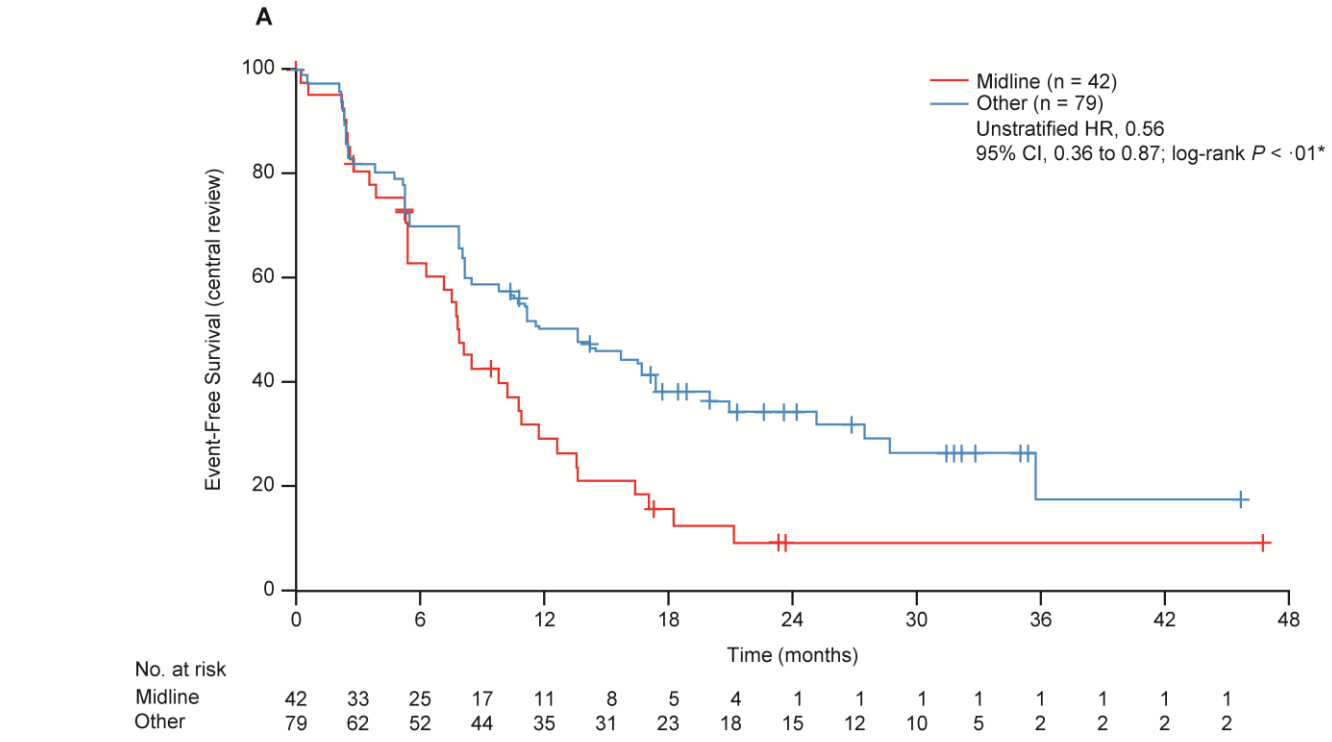
Neuropsychological Function

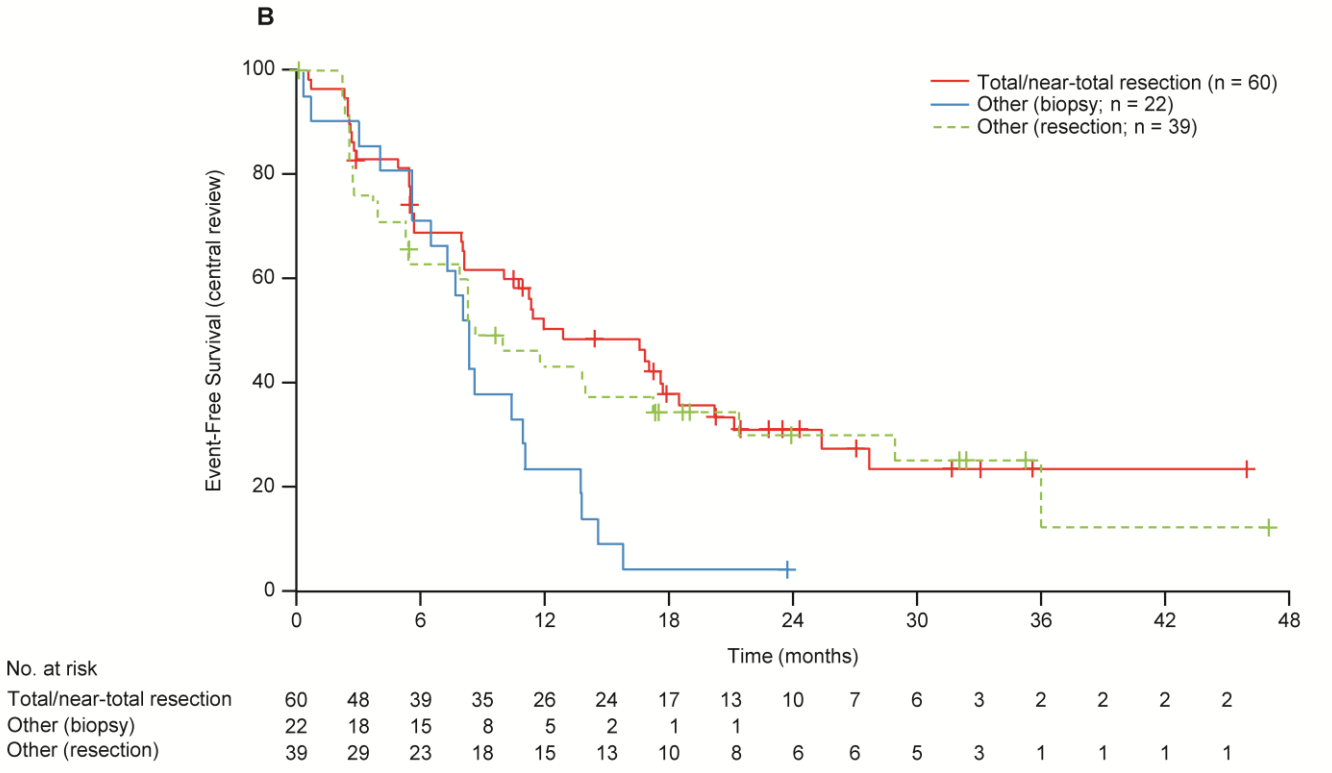
Neuropsychological function assessments were available for more than half of the patients during the treatment period, but availability dropped significantly for the follow-up period due to death and withdrawal of consent. At end-of-treatment follow-up visit 1, assessments from 47 patients were available. Overall, patients for whom data were collected had normal cognitive function, an absence of motor deficit dysfunction, no seizures, absence of raised intracranial pressure, and normal speech and language.

Prognostic Factors

Age, HGG grade (according to the WHO 2007 guidelines), and MGMT promoter status were not associated with an impact on EFS. Midline tumor location, biopsy only, and histone mutation at position K27M were associated with lower EFS (Appendix Figure A1, online only). Multivariate Cox proportional hazards analysis revealed no significant association between potential baseline prognostic factors and EFS, but midline tumor location (*v* other) and a histone mutation at position G34 (*v* no mutation) were associated with poorer overall survival (Appendix Table A2, online only).

Appendix Fig A1. Impact of tumor location (A) and extent of resection (B) on CRRC-assessed event-free survival. CI, confidence interval; CRRC, Central Radiology Review Committee; HR, hazard ratio.





Appendix Table A1. Summary of Sensitivity Analyses on CRRC-Assessed EFS

Sensitivity Analysis	RT/TMZ (n = 59)	BEV+RT/TMZ (n = 62)
Unstratified analysis		
Median, months	11.8	8.2
HR (95% CI)	1.18 (0.77 to 1.81)	
Censoring for new anticancer therapy		
Median, months	11.8	8.2
HR (95% CI)	1.44 (0.90 to 2.30)	
Censoring for treatment discontinuation		
Median, months	16.4	14.5
HR (95% CI)	1.41 (0.80 to 2.49)	

Abbreviations: BEV, bevacizumab; CI, confidence interval; CRRC, Central Radiology Review Committee; EFS, event-free survival; HR, hazard ratio; RT, radiotherapy; TMZ, temozolomide.

Appendix Table A2. Multivariate Cox Proportional Hazards Analysis of the Impact of Prognostic Factors on CRRC-Assessed EFS and OS

		Effect Size	HR	SE	*P* Value
CRRC-assessed EFS					
Treatment	BEV+RT/TMZ	0.047	1.048	0.287	0.8690
Age group	3 to < 6 years	−0.091	0.913	0.517	0.8603
	6 to < 13 years	0.331	1.393	0.299	0.2675
WHO-defined grade of HGG	IV	0.332	1.393	0.315	0.2928
Resection	Other	0.075	1.078	0.298	0.8010
Sex	Female	−0.215	0.806	0.264	0.4151
Tumor location	Midline	0.595	1.812	0.485	0.2205
Histone status	Mutation at position G34	0.724	2.062	0.444	0.1027
	Mutation at position K27	0.318	1.375	0.510	0.5325
OS					
Treatment	BEV+RT/TMZ	−0.133	0.876	0.332	0.6900
Age group	3 to < 6 years	−0.269	0.764	0.606	0.6571
	6 to < 13 years	0.618	1.856	0.365	0.0899
WHO-defined grade of HGG	IV	0.204	1.226	0.360	0.5719
Resection	Other	0.228	1.256	0.339	0.5002
Sex	Female	−0.170	0.844	0.306	0.5792
Tumor location	Midline	1.274	3.576	0.545	**0.0193**

Histone status	Mutation at position G34	1.167	3.212	0.498	**0.0191**
	Mutation at position K27	0.067	1.069	0.547	0.9033

Covariates: treatment (*v* RT/TMZ), age group (*v* 13 to < 18 years), WHO-defined grade of HGG (*v* III), resection complete or biopsy (*v* total or near-total resection), sex (*v* male), tumor location (*v* other), histone status (*v* no mutation).

Abbreviations: BEV, bevacizumab; CRRC, Central Radiology Review Committee; EFS, event-free survival; HGG, high-grade glioma; HR, hazard ratio; OS, overall survival; RT, radiotherapy; SE, standard error; TMZ, temozolomide; WHO, World Health Organization.