Neuro-Oncology

Survival effect of first- and second-line treatments for patients with primary glioblastoma: a cohort study from a prospective registry, 1997–2010

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Background. Prospective follow-up studies of large cohorts of patients with glioblastoma (GBM) are needed to assess the effectiveness of conventional treatments in clinical practice. We report GBM survival data from the Brain Cancer Register of the Fondazione Istituto Neurologico Carlo Besta (INCB) in Milan, Italy, which collected longitudinal data for all consecutive patients with GBM from 1997 to 2010.

Methods. Survival data were obtained from 764 patients (aged>16 years) with histologically confirmed primary GBM who were diagnosed and treated over a 7-year period (2004–2010) with follow-up to April 2012 (cohort II). Equivalent data from 490 GBM patients diagnosed and treated over the preceding 7 years (1997–2003) with follow-up to April 2005 (cohort I) were available for comparison. Progression-free survival (PFS) was available from 361 and 219 patients actively followed up at INCB in cohorts II and I, respectively.

Results. Survival probabilities were 54% at 1 year, 21% at 2 years, and 11% at 3 years, respectively, in cohort II compared with 47%, 11%, and 5%, respectively, in cohort I. PFS was 22% and 12% at 1 year in cohorts II and I. Better survival and PFS in cohort II was significantly associated with introduction of the Stupp protocol into clinical practice, with adjusted hazard ratios (HRs) of 0.78 for survival and 0.73 for PFS, or a 22% relative decrease in the risk of death and a 27% relative decrease in the risk of recurrence. After recurrence, reoperation was performed in one-fifth of cohort I and in one-third of cohort II but was not effective (HR, 1.05 in cohort I and 1.02 in cohort II). Second-line chemotherapy, mainly consisting of nitrosourea-based chemotherapy, temozolomide, mitoxantrone, fotemustine, and bevacizumab, improved survival in both cohorts (HR, 0.57 in cohort I and 0.74 in cohort II). Radiosurgery was also effective (HR, 0.52 in cohort II).

Conclusions. We found a significant increase in overall survival, PFS, and survival after recurrence after 2004, likely due to improvements in surgical techniques, introduction of the Stupp protocol as a first-line treatment, and new standard protocols for second-line chemotherapy and radiosurgery after tumor recurrence. In both cohorts, reoperation after tumor recurrence did not improve survival.

Keywords: glioblastoma, surgery, survival analysis, treatments, treatment effectiveness.

Glioblastoma multiforme (GBM) (World Health Organization grade IV astrocytoma)[1] is the most common primary malignant brain tumor in adults, accounting for 50%–60% of all incident cases of gliomas and having the worst prognosis of all gliomas. In Europe and North America, the incidence of GBM is 2–3 new cases per 100 000 people per year.[2] GBM incidence peaks between ages 45 and 70 years, and the disease occurs more often in males.[3,4] Well-established prognostic and predictive factors for survival are age, functional status on hospital admission as measured by the Karnofsky Performance Scale (KPS), tumor

extent, and surgical resection of the tumor.[3,5,6] The Stupp protocol was approved by regulatory agencies as first-line treatment for GBM after a clinical trial established that temozolomide, concomitant with radiotherapy and then as maintenance treatment, improved survival and progression-free survival (PFS).[7] The Stupp protocol is now the standard treatment for patients with GBM, and several studies have confirmed the beneficial effects of this first-line regimen.[6,8,9]

Median survival for GBM patients after recurrence is ~6 months.[10] Bevacizumab alone or in combination with chemotherapy had been shown to be effective against recurrent GBM,[11,12] which led to its accelerated approval in May 2009 for recurring patients.[13] Increased survival after recurrence has also been reported for patients given salvage radiosurgery.[14,15]

Conversely, the benefit of reoperation is still under discussion and remains controversial. While some retrospective studies did not report an increase in survival of patients with recurrent GBM following second surgery,[8,16,17] other studies have reported a survival benefit after reoperation, although this was mainly limited to young patients with high KPS scores.[5,18–21]

In newly diagnosed GBM, methylation of the O6-methylguanine-DNA methyltransferase (MGMT) promoter has been shown to predict response to alkylating agents such as temozolomide.[22] A recent meta-analysis also suggested that MGMT silencing is a predictive marker that benefits patients who receive chemotherapy as a component of adjuvant treatment.[23]

Here, we report the results of a comparison of survival and PFS between 2 cohorts of patients with primary newly diagnosed GBM treated at the Fondazione Istituto Neurologico Carlo Besta (INCB), a tertiary-care institution in Milan, Italy.

Materials and Methods

Patients

The INCB Cancer Registry was used to prospectively enroll all consecutive patients aged 16 years and older with a new diagnosis of histologically confirmed primary GBM. Patients in cohort I[17] (January 1997–December 2003) were followed up to April 2005, and those in cohort II (January 2004–December 2010) were followed up to April 2012. Secondary GBM patients with previous histological or radiological diagnoses of low-grade or anaplastic astrocytoma (WHO grade II or III astrocytoma) were excluded. Pathological diagnosis was performed by 2 neuropathologists at INCB in accordance with WHO guidelines.[1] All patients provided written informed consent to undergo surgery and chemoradiotherapy. Each was

Table 1. Characteristics of patients at baseline by cohort

Factor	Cohort I ($n = 490$)[a] No. of Patients (%)	Cohort II ($n = 764$)[b] No. of Patients (%)	P value
Sex			
Male	295 (60.2%)	512 (67.0%)	.01[c]
Female	195 (39.8%)	252 (33.0%)	
Age (years)			
≤50	127 (25.9%)	162 (21.2%)	.07[c]
51–65	234 (47.8%)	364 (47.6%)	
>65	129 (26.3%)	238 (31.2%)	
KPS[d]			
≤70	182 (37.9%)	157 (20.7%)	<.01[c]
>70	298 (62.1%)	603 (79.3%)	
Tumor extent			
Multiple lobes	226 (46.1%)	343 (45.1%)	.73[c]
Single lobe	264 (53.9%)	417 (54.9%)	
Surgery			
Biopsy only	70 (14.3%)	45 (5.9%)	<.01[c]
Surgical resection	420 (85.7%)	719 (94.1%)	
Treatment schedule[d]			
Radiotherapy + chemotherapy[e]	304 (67.1%)	229 (31.3%)	<.01[c]
Stupp protocol	0	336 (45.9%)	
Other protocol[f]	149 (32.9%)	167 (22.8%)	
	Median (95% CI)	Median (95% CI)	
Follow-up (months)	59.0 (95% CI, 38.7–73.5)	46.2 (95% CI, 35.3–54.6)	.07[g]

[a]Histologically confirmed primary glioblastoma newly diagnosed between 1997 and 2003 and followed to April 2005.
[b]Histologically confirmed primary glioblastoma newly diagnosed between 2004 and 2010 and followed to April 2012.
[c]Chi-square test.
[d]Sum does not equal total because some values are missing.
[e]Other than Stupp protocol.
[f]Could be radiotherapy only, chemotherapy only, or no treatment.
[g]Log-rank test.

also asked to give written consent for personal data to be used anonymously for research purposes. The study was approved by the review board of INCB.

Outcomes

Death certificates were collected from municipal offices yearly. Survival was defined as time from the first surgery to death or the end of follow-up (April 30, 2005, for cohort I or April 30, 2012, for cohort II). PFS was defined as time from first surgery to first evidence of recurrence or death, or the end of follow-up. Tumor recurrence was defined as the appearance of new lesions, a 25% increase in tumor extent on CT or MRI, worsening of clinical/neurological condition, or increased need for corticosteroids as defined by the Macdonald criteria.[24] The updated response criteria published in April 2010 (RANO criteria)[25] were not applicable to our study. Survival after tumor recurrence was defined as time from tumor recurrence to death or end of follow-up.

Predictors of Survival

Sex, age, preoperative KPS score (assessed on the day before surgery), tumor extent (single lobe vs multiple lobes), surgery (resection vs biopsy), and treatment schedule (Stupp protocol, radiotherapy plus chemotherapy schedules differing from Stupp protocol, radiotherapy only, chemotherapy only, or no treatment) were included in the survival model as predictors. First-line chemotherapy was defined as any chemotherapy agent used consecutively for at least 1 month.

Sex, age, time to recurrence (≥ 9 vs <9 months after the first surgery), second-line chemotherapy (yes vs no), second-line radiotherapy (yes vs no), and second surgery (yes vs no) were included in the analysis of survival after tumor recurrence.

MGMT status (methylated vs unmethylated) was tested as a predictor of survival together with sex, age, KPS, tumor extent, and surgery. The methylation status of the *MGMT* promoter was assessed using methylation-specific PCR.[26]

Data

Data from both cohorts were compared to assess the predictive effect of first-line treatments on overall survival (OS) and PFS and the effects of reoperation and second-line treatments on survival after recurrence. Analyses on PFS and survival after tumor recurrence were performed only on patients actively followed-up at INCB. Survival by *MGMT* status was analyzed on a subset of cohort II patients for whom test results were available.

Statistical Analysis

A description of baseline patient characteristics was provided in terms of percentages, and differences between the cohorts were evaluated using the chi-square test for frequency and the log-rank test for median comparisons. OS and PFS were estimated using the Kaplan-Meier method and Cox proportional hazards model.[27] The Kaplan-Meier method was used to obtain survival curves, survival medians, and probabilities at different time points (1, 2, and 3 years for OS and 6, 12, and 18 months for PFS). The Cox proportional hazards models provided hazard ratios (HRs) as relative risk estimates of survival for given combinations of prognostic and predictive factors(ie., sex, age, KPS, tumor extent, surgery, and treatment schedule). Survival after recurrence was estimated using the Kaplan-Meier method and a time-dependent Cox model that included sex, age, time to recurrence (cut-off = 9 months), reoperation, second-line chemotherapy, and second-line radiotherapy.
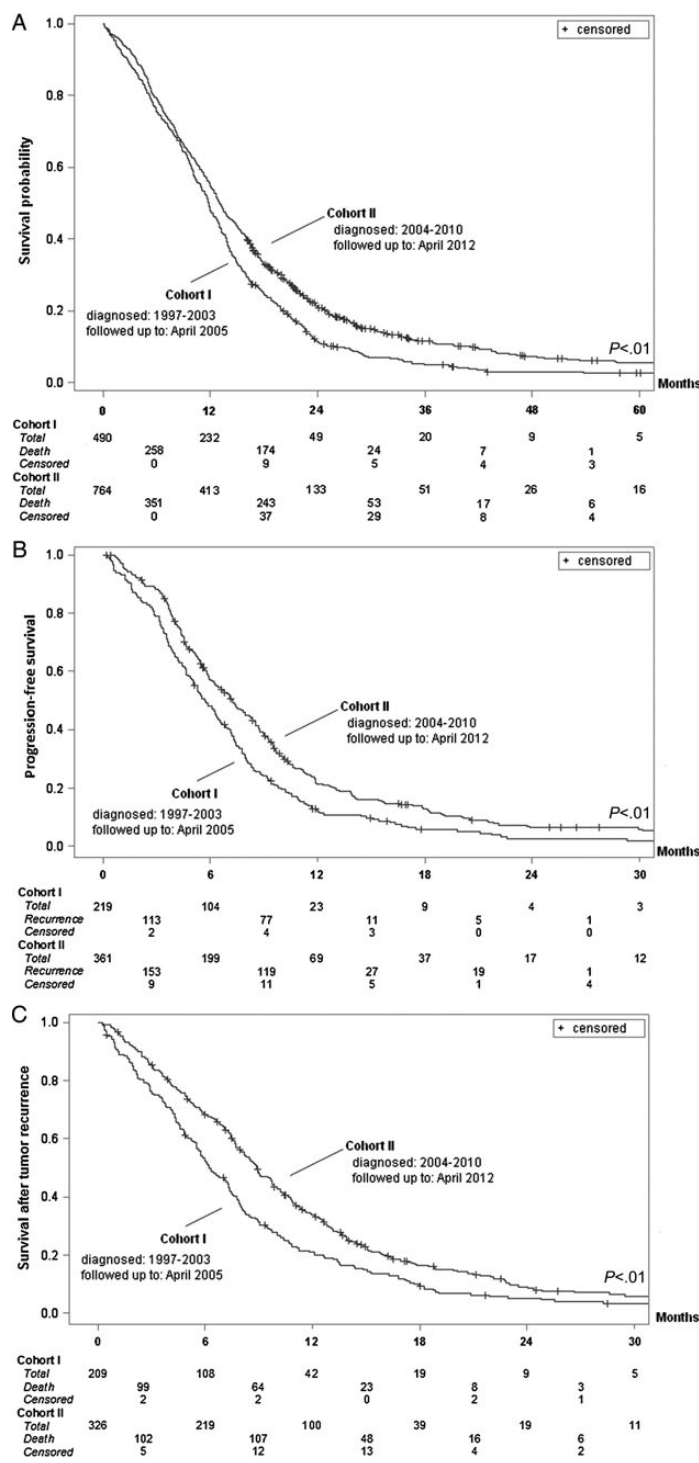

Fig. 1. Kaplan-Meier curves of (A) overall survival, (B) progression-free survival, and (C) survival after tumor recurrence by cohort: histologically confirmed primary glioblastoma newly diagnosed between 2004 and 2010 and followed up to April 2012 (cohort II) vs confirmed primary glioblastoma newly diagnosed between 1997 and 2003 and followed up to April 2005 (cohort I). Analyses on progression-free survival and survival after tumor recurrence were performed only on patients actively followed at Fondazione Istituto Neurologico Carlo Besta.

Table 2. Multivariable Cox proportional hazards modeling of overall survival by cohort

Factor	Cohort I (n = 445)[a]		Cohort II (n = 725)[b]	
	No. of Events/ No. of Patients	HR (95% CI)	No. of Events/ No. of Patients	HR (95% CI)
Sex				
Male	259/274	1	435/486	1
Female	162/171	0.85 (0.69–1.05)	200/239	0.81 (0.68–0.96)
Age (years)[c]	–	1.02 (1.01–1.03)	–	1.02 (1.01–1.03)
KPS				
≤70	158/162	1	142/148	1
>70	263/283	0.76 (0.62–0.94)	493/577	0.58 (0.48–0.70)
Tumor extent				
Multiple lobes	192/205	1	305/329	1
Single lobe	229/240	0.82 (0.67–1.02)	330/396	0.79 (0.67–0.92)
Surgery				
Biopsy only	59/60	1	42/42	1
Surgical resection	362/385	0.55 (0.40–0.74)	593/683	0.62 (0.44–0.85)
Treatment schedule				
Radiotherapy + chemotherapy[d]	276/298	1	211/228	1
Stupp protocol	–	–	263/333	0.78 (0.65–0.94)
Other protocol[e]	145/147	2.75 (2.18–3.47)	161/164	2.66 (2.12–3.34)

[a]Histologically confirmed primary glioblastoma newly diagnosed between 1997 and 2003 and followed to April 2005. Forty-five cases with missing data for one or more covariates were excluded from the analysis.
[b]Histologically confirmed primary glioblastoma newly diagnosed between 2004 and 2010 and followed to April 2012. Thirty-nine cases with missing data for one or more covariates were excluded from the analysis.
[c]Continuous variable.
[d]Other than Stupp protocol.
[e]Could be radiotherapy only, chemotherapy only, and no treatment.

Results

Patients

The number of patients with GBM admitted to INCB increased by 56%, from 490 between 1997 and 2003 to 764 between 2004 and 2010. Survival status was verified for all patients, and the completeness index of follow-up was 100%.[28] Patient characteristics are summarized in Table 1. In cohorts I and II, the ratio of males to female was 1:0.7 and 1:0.5 (P = .01), respectively, with 26.3% and 31.2% of patients over 65 years of age (P = .07), KPS score >70 in ~60% and 80% (P < .01), and tumor confined to single lobe in more than 50% of the patients in both cohorts (P = .73). Most patients (85.7% in cohort I and 94.1% in cohort II; P < .01) underwent surgical resection; the remaining patients received biopsy only. Patients who received a combination of radiotherapy and chemotherapy (different from the Stupp protocol) were 67.1% in cohort I and 31.3% in cohort II; none in cohort I and 45.9% in cohort II received the Stupp protocol; 32.9% in cohort I and 22.8% in cohort II received radiotherapy only, chemotherapy only, or no treatment (P < .01 for treatment schedule comparison between the 2 cohorts). The most widely used first-line chemotherapies were nitrosourea-based chemotherapy (74%) in cohort I, and temozolomide (almost 90%) in cohort II. The postoperative radiotherapy schedule was the same in both cohorts: external beam radiation therapy of 60 Gy (fractionated into 1.8–2 Gy daily fractions) targeting the enhancing portion of the tumor and a 2–3 cm margin.

Follow-up ranged from 16.4 to 91.2 months in cohort I and from 15.9 to 77.6 months in cohort II. Median follow-up was 59.0 months in cohort I and 46.2 months in cohort II (P = .07).

Comparison of Survival for Patients in Cohorts I and II

Fig. 1 shows Kaplan-Meier curves for OS (A), PFS (B), and survival after tumor recurrence (C) for both cohorts. There was a statistically significant difference (log-rank tests, P < .01) between the 2 cohorts in the 3 survival curves.

At the end of follow-up, 24 (4.9%) cohort I and 91 (11.9%) cohort II patients were still alive. In cohort I, the median OS was 11.7 months (95% CI, 10.8–12.5 months), and survival probabilities were 47% (95% CI, 43%–52%) at 1 year, 11% (95% CI, 8%–14%) at 2 years, and 5% (95% CI, 3%–7%) at 3 years. The corresponding estimates for cohort II were median OS of 12.9 months (95% CI, 12.2–13.7 months), and survival probabilities of 54% (95% CI, 52%–56%) at 1 year, 21% (95% CI, 20%–22%) at 2 years, and 11% (95% CI, 10%–13%) at 3 years. Table 2 shows the prognostic and predictive factors included in the OS analysis with corresponding HRs for death, estimated using a multivariable Cox proportional hazards model. Age, KPS, tumor extent, surgery, and treatment schedule were prognostic or predictive factors of OS in both cohorts. In cohort II, a

Table 3. Multivariable Cox proportional hazards model for progression-free survival by cohort

Factor	Cohort I (n = 206)[a]		Cohort II (n = 353)[b]	
	No. of Events/ No. of Patients	HR (95% CI)	No. of Events/ No. of Patients	HR (95% CI)
Sex				
Male	118/123	1	218/238	1
Female	80/83	0.87 (0.65–1.17)	101/115	0.84 (0.66–1.07)
Age (years)[c]	–	1.02 (1.01–1.03)	–	1.00 (0.99–1.01)
KPS				
≤70	66/67	1	39/40	1
>70	132/139	0.61 (0.45–0.83)	280/313	0.71 (0.51–1.00)
Tumor extent				
Multiple lobes	92/94	1	124/134	1
Single lobe	106/112	0.75 (0.55–1.02)	195/219	0.83 (0.66–1.05)
Surgery[d]				
Biopsy only	21/22	1	–	–
Surgical resection	177/184	0.65 (0.40–1.07)	–	–
Treatment schedule				
Radiotherapy + chemotherapy[e]	163/166	1	152/155	1
Stupp protocol	–	–	151/181	0.73 (0.58–0.92)
Other protocol[f]	35/40	1.82 (1.23–2.70)	16/17	2.35 (1.40–3.97)

[a]Histologically confirmed primary glioblastoma newly diagnosed between 1997 and 2003 and actively followed to April 2005 at INCB. Thirteen cases had missing data for one or more covariates and were excluded from the analysis.
[b]Histologically confirmed primary glioblastoma newly diagnosed between 2004 and 2010 and actively followed to April 2012 at INCB. Eight cases with missing data for one or more covariates were excluded from the analysis.
[c]Continuous variable.
[d]HR for surgery in cohort II was not calculated because there were too few cases in the reference category (4 of 353 patients had biopsy only in cohort II).
[e]Other than Stupp protocol.
[f]Could be radiotherapy only, chemotherapy only, and no treatment.

significant relative reduction of more than 20% (HR = 0.78; 95% CI, 0.65–0.94) in the risk of death was observed for patients who received the Stupp protocol, compared with those who received radiotherapy and chemotherapy schedules other than the Stupp protocol.

Based on 219 and 361 patients actively followed at INCB in cohorts I and II, respectively, 10 (4.6%) cohort I and 35 (9.7%) cohort II patients were still free from recurrence at the end of follow-up. Median PFS was 5.7 months (95% CI, 5.0–6.4 months), and PFS probabilities were 48% (95% CI, 42%–55%) at 6 months, 12% (95% CI, 8%–16%) at 12 months, and 6% (95% CI, 3%–9%) at 18 months, in cohort I. The corresponding estimates in cohort II were a median PFS of 7.2 months (95% CI, 6.4–8.2 months), and survival probabilities of 57% (95% CI, 52%–62%) at 6 months, 22% (95% CI, 18%–26%) at 12 months, and 13% (95% CI, 9%–17%) at 18 months. Table 3 shows the prognostic and predictive factors included in the PFS analysis with corresponding HRs for recurrence, estimated by multivariable Cox proportional hazards model. A significant relative reduction of about 30% (HR = 0.73; 95% CI, 0.58–0.92) in the risk of recurrence was found for patients who received the Stupp protocol, compared with those who received radiotherapy and chemotherapy treatment schedules different from the Stupp protocol.

Two-hundred nine and 326 patients had tumor recurrence in cohorts I and II, respectively. Ten (4.8%) cohort I and 44 (13.5%)

cohort II patients were still alive at the end of follow-up. In cohort I, median survival after recurrence was 6.2 months (95% CI, 5.5–7.3 months), and survival probabilities were 52% (95% CI, 46%–59%) at 6 months, 21% (95% CI, 15%–27%) at 12 months, and 10% (95% CI, 5%–14%) at 18 months. The corresponding estimates in cohort II were median survival after tumor recurrence of 8.9 months (95% CI, 8.0–9.8 months), and survival probabilities of 69% (95% CI, 63%–74%) at 6 months, 34% (95% CI, 29%–39%) at 12 months, and 17% (95% CI, 12%–21%) at 18 months. Table 4 shows the prognostic and predictive factors included in the survival after recurrence analysis, with the corresponding HRs for death estimated using a multivariable time-dependent Cox model. The analysis revealed a significant relative reduction in risk of death after recurrence for patients receiving second-line chemotherapy (HR = 0.57; 95% CI, 0.41–0.79 for cohort I and HR = 0.74; 95% CI, 0.56–0.97 for cohort II), compared with patients who did not receive second-line chemotherapy. Second-line chemotherapy mainly consisted of nitrosourea-based chemotherapy (49% in cohort I and 33% in cohort II), temozolomide (21% in cohort I and 26% in cohort II), mitoxantrone (19% in cohort I and 10% in cohort II), fotemustine (18% in cohort II only), and bevacizumab (5% in cohort II, only). The risk of death after recurrence associated with second-line radiotherapy was lower for patients in cohort II (HR = 0.52; 95% CI, 0.37–0.75) than those in cohort I (HR = 0.94; 95% CI, 0.65–1.37).

Table 4. Multivariable time-dependent Cox model for survival after recurrence by cohort

Factor	Cohort I (n = 203)[a]		Cohort II (n = 303)[b]	
	No. of Events/ No. of Patients	HR (95% CI)	No. of Events/ No. of Patients	HR (95% CI)
Sex				
Male	115/121	1	186/206	1
Female	78/82	0.74 (0.55–0.99)	80/97	0.75 (0.57–0.98)
Age (years)[c]	–	1.01 (1.00–1.02)	–	1.02 (1.01–1.04)
Time to recurrence[d]				
<9 months	154/158	1	185/198	1
≥9 months	39/45	0.76 (0.53–1.09)	81/105	0.65 (0.49–0.87)
Second-line chemotherapy[e]				
No	113/118	1	107/117	1
Yes	80/85	0.57 (0.41–0.79)	159/186	0.74 (0.56–0.97)
Second-line radiotherapy[f]				
No	156/164	1	230/255	1
Yes	37/39	0.94 (0.65–1.37)	36/48	0.52 (0.37–0.75)
Second surgery[g]				
No	155/163	1	182/205	1
Yes	38/40	1.05 (0.71–1.54)	84/98	1.02 (0.77–1.34)

[a]Histologically confirmed primary glioblastoma newly diagnosed between 1997 and 2003 and actively followed to April 2005 at INCB. Six cases with missing values of one or more covariates were excluded from the analysis.
[b]Histologically confirmed primary glioblastoma newly diagnosed between 2004 and 2010 and actively followed to April 2012 at INCB. Twenty-three cases with missing values in one or more covariates were excluded from the analysis.
[c]Continuous variable.
[d]Defined as time from first surgery to tumor recurrence. Included in model as potential confounder only.
[e]Defined as first chemotherapy regimen received after tumor recurrence; consisted of procarbazine, temozolomide, fotemustine, mitoxantrone, and bevacizumab in both cohorts. Included in the model as time-dependent covariate.
[f]Defined as a radiotherapy received after tumor recurrence; consisted of radio-immune therapy in cohort I and radiosurgery in cohort II. Included in the model as time-dependent covariate.
[g]Defined as the first surgery performed after tumor recurrence. Included in the model as time-dependent covariate.

Second-line radiotherapy consisted of radio-immune therapy in cohort I and of radiosurgery in cohort II. Reoperation after recurrence was not associated with increased survival in either cohort (HR = 1.05; 95% CI, 0.71–1.54 in cohort I and HR = 1.02; 95% CI, 0.77–1.34 in cohort II). Sensitivity analyses performed excluding patients who died within a month of reoperation did not change the results (HR = 0.97; 95% CI, 0.66–1.44; n = 201 in cohort I and HR = 0.96; 95% CI, 0.73–1.28; n = 299 in cohort II).

MGMT Methylation Analysis

Data on *MGMT* methylation were available for 240 (41%) of cohort II patients who underwent chemotherapy: 75 (31%) were methylated, and 165 (69%) were unmethylated. Fig. 2 shows Kaplan-Meier curves of OS for methylated and unmethylated patients. At the end of follow-up (median OS, 16.8 months), 21 of the 75 methylated (28%) and 14 of the 165 (8%) unmethylated patients were still alive. Survival probabilities were 81% (95% CI, 73%–90%) and 68% (95% CI, 61%–75%) at 1 year, 56% (95% CI, 45%–68%), and 20% (95% CI, 14%–26%) at 2 years, 37% (95% CI, 24%–48%) and 8% (95% CI, 4%–12%) at 3 years, for methylated and unmethylated patients, respectively. Survival for methylated patients was significantly longer than for unmethylated patients, both in terms

of median OS (26.7 months; 95% CI, 21.8–34.5 months for methylated vs 15.7 months, 95% CI, 13.3–16.8 months for unmethylated; log-rank test, P < .01), and in terms of HR adjusted for sex, age, tumor extent, KPS, and surgery (HR = 0.45; 95% CI, 0.33–0.63; n = 238).

Discussion

OS in the 2004–2010 cohort was significantly better than that in the 1997–2003 cohort, suggesting a relationship to the improvements in surgical techniques and new protocols for first-line chemotherapy and second-line radiosurgery that had become available for cohort II. The number of patients with GBM admitted to INCB was also significantly higher in the later period. The number of operating rooms available for neurosurgery increased from 2 to 4 starting in 2005. Furthermore, the number of GBM patients presenting at INCB for treatment has increased over the last 7–8 years, so that the Institute now treats more patients than any other center in Italy. INCB has always been a reference center for neurosurgery in Italy and is now one of the relatively few centers designated by the Italian Health Ministry for such treatment. Finally, the shorter median length of stay of cohort II than cohort I (7 vs 12 days) probably reflects increasing concern for costs but
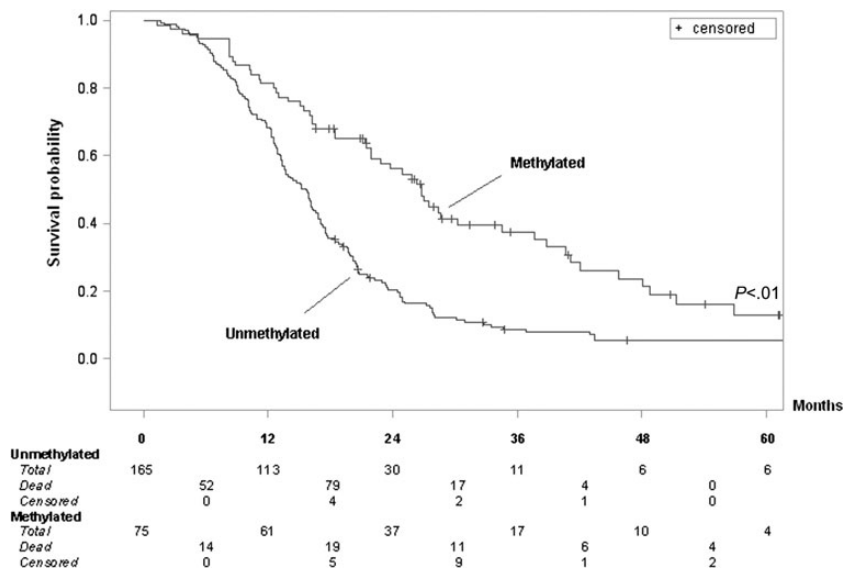
Fig. 2. Kaplan-Meier curves of overall survival by *MGMT* (O6-methylguanine-DNA methyltransferase) methylation status. Two-hundred forty patients with histologically confirmed primary glioblastoma newly diagnosed between 2004 and 2010 and followed up to April 2012 underwent chemotherapy and provided information on *MGMT* methylation status. Survival by *MGMT* status was analyzed on a subset of cohort II patients for whom *MGMT* status results were available.

also improvement in patient care, which in turn made volume growth possible.

The 2 cohorts did not differ significantly for 2 established survival predictors (age and tumor extent) but did differ for presurgery functional status, which was better in cohort II patients. Cohort II patients did not undergo first surgery earlier than those in cohort I, since median times from radiologic diagnosis to surgery were indistinguishable (27 days for cohort I vs 26 days for cohort II); however, earlier diagnosis in cohort II is possible, though we have no data to support this. A possible reason why a greater proportion of cohort II patients underwent surgical resection is that surgical techniques had improved considerably for this cohort following the introduction of neuronavigation tools, including ultrasound for real-time intraoperative navigation. To avoid biases due to these between-cohort differences, we analyzed the 2 cohorts separately using multivariate methods to adjust for potential confounding.

We found consistent results in terms of prognostic and predictive factors in the 2 cohorts that indicated a reduction of more than 20% in the relative risk of death and tumor recurrence after the introduction of the Stupp protocol as first-line treatment. A similar positive effect of the Stupp protocol has been reported by other studies.[8,9] However, compared with the Stupp trial,[7] we had slightly worse survival (median OS of 13.1 months in our patients recruited after 2005; 14.6 months in Stupp), probably because our study was conducted in clinical practice, whereas patients included in clinical trials are selected, have greater treatment compliance, and typically survive longer.[29,30] Two observational studies conducted in Northern Europe[31,32] and a US study[33] also had similar results to ours: all compared patients in the pre- and post-temozolomide era and found a favorable effect of the Stupp protocol with median OS of 9.7 to 12.0 months.

In our study, second-line chemotherapy (mainly consisting of nitrosourea-based chemotherapy, temozolomide, mitoxantrone,

fotemustine, and bevacizumab), and radiosurgery were associated with lower relative risk of death after tumor recurrence. These findings, together with those of previous studies,[14,15,34–37] suggest that second-line treatments can prolong the survival of patients with recurrent GBM. By contrast, we found that reoperation after tumor recurrence did not prolong survival in either cohort. Other studies have also found that survival after recurrence was not noticeably better in patients undergoing further surgical resection.[8,16,17,38,39] Other studies again have found that second surgery after recurrence does improve survival.[5,18–21] However, these studies reported only univariate analyses, raising the suspicion that the survival advantage may have been affected by confounders. Indications for treatment and treatment administration are often not standardized in observational studies, and confounding by indication can lead to bias.[40] In fact, it has been reported that patient selection for second surgery can be influenced by favorable prognostic factors such as young age, high preoperative KPS score ($\geq$80), small tumor volume ($\leq$50 cm^3), and latency between first surgery and tumor recurrence of $\geq$6 months.[41–43] In order to reduce the influence of selection bias, we analyzed data using a multivariable model that adjusted for several potential confounding factors.

In line with the results of a recent meta-analysis,[23] we found that MGMT methylation status was a statistically significant independent predictor of OS in patients with primary GBM, with a >50% lower relative risk of death for methylated patients, compared with unmethylated patients. These findings therefore constitute additional evidence that this marker is useful in clinical decision-making and that it may reduce treatment costs and toxicity in specific patient subgroups by withholding ineffective medication.[44]

Drawing conclusions on the predictive effect of treatments from cohort studies is difficult because the effects of treatments are often overestimated by indication, selection, performance,

and detection bias.[45,46] However, applying a multivariable approach in design and analysis, as we did, to determine outcome predictors boosts confidence in the reliability of the estimates thus derived. Randomized trials overcome selection bias and confounding but have limited generalizability because of strict eligibility criteria, low recruitment levels, or high levels of nonconsent,[46] leading to findings inconsistent with daily practice.[47] Provided putative predictive factors are interpreted cautiously, our findings provide useful support for decision-making in clinical practice as well as indications for future research. Since clinical trials to resolve remaining uncertainties about the utility of reoperation following tumor recurrence are difficult to conduct, this important clinical question should be resolved by carrying out large prospective well-conducted cohort studies.

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