Turk Neurosurg 31(4):641-653, 2021

Original Investigation

DOI: 10.5137/1019-5149.JTN.33332-20.3

Received: 15.12.2020
Accepted: 08.03.2021

Published Online: 18.03.2021

Predictors of Survival in Turkish Patients with Primary Glioblastoma

Emre BILGIN[1], Berna BOZKURT DUMAN[2], Suleyman ALTINTAS[3], Timucin CIL[2], Yurdal GEZERCAN[1], Ali Ihsan OKTEN[1]

[1]Adana City Education and Research Hospital, Department of Neurosurgery, Adana, Turkey
[2]University of Health Sciences Adana City Education and Research Hospital, Department of Medical Oncology, Adana, Turkey
[3]Adana City Education and Research Hospital, Department of Pathology, Adana, Turkey

Corresponding author: Emre BILGIN ✉ dremreblgn@gmail.com

ABSTRACT

AIM: An increasing number of biomarkers of primary glioblastoma (GBM) have recently been described. We aimed to investigate the biological and clinical factors that affect survival in Turkish patients with primary GBM.

MATERIAL and METHODS: The clinical and demographic data of all patients with primary GBM diagnosed between 2007 and 2016 were evaluated. In all the patients' pathological specimens, O6 methylguanine-DNA methyltransferase (MGMT) methylation and isocitrate dehydrogenase (IDH) 1 mutation were detected retrospectively by immunohistochemistry. Kaplan-Meier survival analysis, log-rank test, and multivariate analyses of the Cox hazard proportional model for all the variables were performed using the SPSS statistical package. The treatment details and other patient-related factors were identified, and their correlations were analyzed.

RESULTS: We enrolled 137 primary GBM patients to the study. Median progression free survival (PFS) was 8.57 months (95% CI:6.8-9.5) and median overall survival (OS) was 12 months (95% CI:10.8-13.3). IDH-1 mutations were detected in 21 primary GBMs (15.3%). PFS was 15.43 ± 1.95 months. Survival rates were higher, but no statistically significant difference (p=0.074). MGMT methylation was detected in 40 primary GBMs (29.2%). OS and PFS of MGMT (+) cases were higher than MGMT(-) cases (p=0.001; p=0.001 respectively). Ki67 (%) measurement (10%-90%) average is 32.64 ± 16.56. No statistically significant between higher and lower ki67 levels (p=0.510, p=0.505 respectively). KPS (%) more than 70 at the time of diagnosis statistically significant longer median OS and PFS (p=0.001). PFS and OS were higher in all treatment modalities.

CONCLUSION: The most important factors that affected survival were performance score, MGMT methylation status, systemic oncologic therapy, and IDH mutation in the Turkish population with primary GBM. We demonstrated that MGMT methylation and higher KPS levels were associated with significiantly longer OS and PFS.

KEYWORDS: Primary glioblastoma, Isocitrate dehydrogenase 1 (IDH-1), Mutant, Wild, MGMT methylation

INTRODUCTION

Glioblastoma (GBM) is an aggresive and most common primary malignant brain tumour, usually occurring between 55 and 60 years of age (3). The standard treatment is maximal surgical resection, radiotherapy (RT), and temozolomide (TMZ) therapy (3). Even with maximal therapy, GBM has a high recurrence rate and poor overall survival (OS) ranging from 1 to 2 years (28). Antiangiogenic bevacizumab therapy is frequently used for recurrent disease (6,26). A phase III trial demonstrated the OS advantage of adding concomitant and adjuvant TMZ therapies to the standard RT in patients with GBM (95% confidence interval

Emre BILGIN : 0000-0002-2394-1503
Berna Bozkurt DUMAN : 0000-0003-0295-6295
Suleyman ALTINTAS : 0000-0002-4468-4120
Timucin CIL : 0000-0002-5033-1479
Yurdal GEZERCAN : 0000-0002-4124-2036
Ali Ihsan OKTEN : 0000-0003-0292-201X

[CI], 13.2–16.8 months and 11.2–13.0 months, respectively) (3). In the phase III EF-14 trial, the addition of tumor treating fields to TMZ therapy not only provided a survival advantage (20.9 months vs 16.0 months, 95% CI, 0.53–0.76), but also increased the 5-year survival rates from 5% to 13%. Despite intensive treatment modalities, GBM patients have a poor prognosis, with a median survival time of 14–16 months and 5-year survival rate of 9.8% (22,32).

The World Health Organization (WHO) changed the classification of glioma by incorporating molecular parameters and emphasizing the genotype for diagnosis (37). The WHO divided central nervous system (CNS) tumors into IDH-1 and IDH-2 gene mutations and 1p/19q codeletion in 2016. The citric acid cycle enzyme IDH-1 mutation is responsible for gliomagenesis in 10% of primary GBMs (20). GBM patients with IDH-1 mutation had a better prognosis than those without IDH-1 mutation (5-year survival rate, 93% vs 51%) (14).

The DNA repair enzyme MGMT plays an important role in resistance to TMZ therapy in patients with GBM (4). MGMT methylation has both prognostic and predictive significance in patients with GBM. Epigenetic silencing of the DNA repair enyzme MGMT determines both better outcomes and response to TMZ therapy (4,12).

We aimed to investigate MGMT methylation and IDH mutation, KPS, sex, age, responses to treatment modalities, and survival analysis in 137 patients with primary GBM.

MATERIAL and METHODS

Study Population

Data were obtained from patients with GBM from the University of Health Sciences Turkey, Adana City Education and Research Hospital (UHST-ACERH), between August 2007 and September 2016, for the following variables: IDH mutation, MGMT methylation, KPS, sex, age at diagnosis, and concurrent RT/TMZ therapy. The key criteria for inclusion in the study were as follows: age ≥ 18 years, Eastern Cooperative Oncology Group performance score of 0–1, normal hepatic and renal functions, and sufficient bone marrow reserve. The key exclusion criteria were as follows: another malignancy history, gliosarcomas, major complications after surgery, severe cardiovascular disease, insufficient recovery from toxicities, operation for biopsy only, and subtotal tumor resection, and nationality other than Turkish. All the patients underwent surgery under intratracheal anesthesia in an appropriate position. After craniotomy with dura incision under the guidance of neuronavigation, gross total mass excision was performed. Involved-field RT was performed as a standard component of the initial multimodality therapy in all the patients. The total RT doses was 60 Gy in 2-Gy fractions for GBM. Chemoradiotherapy with TMZ 75 mg/m^2 was admnistered to all the patients after surgery. Patients without progression after chemoradiotherapy received TMZ therapy at a dose of 150–200 mg/m^2 daily for 5 days of the 28-day treatment cycle, thereby completing the adjuvant treatment. Bevacizumab + irinotecan combination therapy was started in the patients who showed progress during follow-up.

The WHO reclassification included molecular testing of IDH mutation for brain tumor. After operation, all the patients were retrospectively asssesed using this classification. IDH mutation and MGMT methylation were detected with immunohistochemistry. The ethics committee of the UHST-ACERH (date: November 21, 2018, no. 25/319) approved the study.

Analysis of IDH-1 Immunohistochemical Results

For immunohistochemistry, 4-µm-thick tissue sections were deparaffinized in xylene and hydrated by immersion in a series of graded ethanol. Microwave antigen retrieval was performed by placing the sections in an epitope retrieval solution (0.01 M citrate buffer, pH 6.0) for 20 minutes; endogenous peroxidase was inhibited by immersing the sections in 0.3% hydrogen peroxide for 10 minutes. The sections were then incubated with IDH-1 antibody. A kit was used following the manufacturer's recommendations in conjunction with an automated staining procedure (24). The samples were counterstained with hematoxylin, dehydrated, mounted, and evaluated under a light microscopic camera (Figure 1A–C).

Analysis of MGMT Methylation Immunohistochemical Results

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue sections. For antigen retrieval, slides were heated at 98–99°C in an ethylenediamine tetra-acetic acid buffer (pH 8.0) for 40 min, followed by cooling in the same buffer for 20 min. Endogenous peroxidases were blocked with 30% H_2O_2 diluted 10 times in phosphate-buffered saline. After washing, the slides were incubated for 45 min at room temperature with the anti-MGMT antibody clone MT3.1 (dilution 1/25). Tumor sections were revealed using a kit, following the manufacturer's instructions. Normal, endothelial, and tumor cells were discriminated on the basis of their morphologies. Normal cells, endothelial cells, and

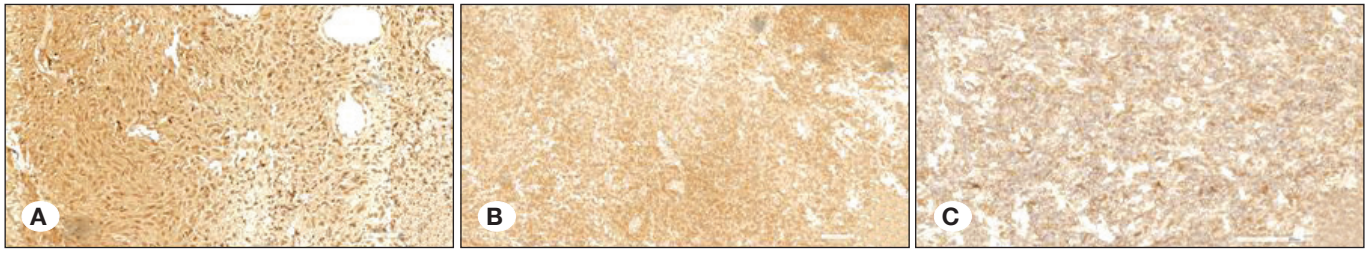

Figure 1: IDH staining by immunohistochemistry x10 **A)** IDH mutant, **B)** IDH NOS, **C)** IDH Wild.

lymphocytes that presented with nuclear immunostaining were not taken into account, and only tumoral cells were quantified. Nuclear immunostaining showed a variable extent of intensity; all the tumoral cells with nuclear immunostaining (high or low intensity) were counted as positive. The percentage of positive cells in the most highly stained areas of each tumor section was determined by counting at least 200 contiguous cells (17).

Statistical Analyses

We used the Number Cruncher Statistical System program for the statistical analyses. Descriptive statistical methods (median, standard deviation, mean, frequency, ratio, maximum value, and minimum value) were used for evaluating the data. A log-rank test and Kaplan-Meier survival analysis were used to evaluate survival. The Fisher-Freeman-Halton exact test was also used. Cox proportional-hazards models were used to evaluate the covariates affecting OS. The significance was set at $p<0.05$.

RESULTS

Demographics and Survival of the Patients

One hundred thirty-seven patients were included in the study, of whom 56.9% (n=78) were male and 43.1% (n=59) were female. The ages ranged from 19 to 91 years, with a mean of 56.42 ± 13.09 years. While 43.1% (n=59) of the patients were aged <55 years, 56.9% (n=78) were aged ≥55 years (Table I). The median PFS was 8.56 months (95% CI, 6.8–9.5), and the median OS was 12 months (95% CI, 10.8–13.3). The median OS was 13 months (95% CI, 5.84–20.16) for the patients aged <55 years and 11.1 months (95% CI, 7.42–14.77) for those older than 55 years (p=0.156). The median OS was 13 years (95% CI, 8.45–17.54) for the women and 11.1 months (95% CI, 7.43–14.76) for the men (p=0.331; Figures 2A, B; 3A, B; 4A, B).

Ki67 and KPS Status and Survival

The Ki67 (%) measurements of the cases ranged from 10% to 90%, with a mean of 32.64% ± 16.56%. Of the patients, 35% (n=48) had Ki67 levels of ≤20% and 65% (n=89) had Ki67 levels >20% (Table I). The differences in PFS and OS between the higher and lower Ki67 levels were not statistically significant (p=0.505 and p=0.510, respectively; Tables II and III; Figure 5A, B).

The KPS (%) measurements of the patients ranged from 60% to 90%, with a mean of 73.5% ± 8.62%. Of the patients,

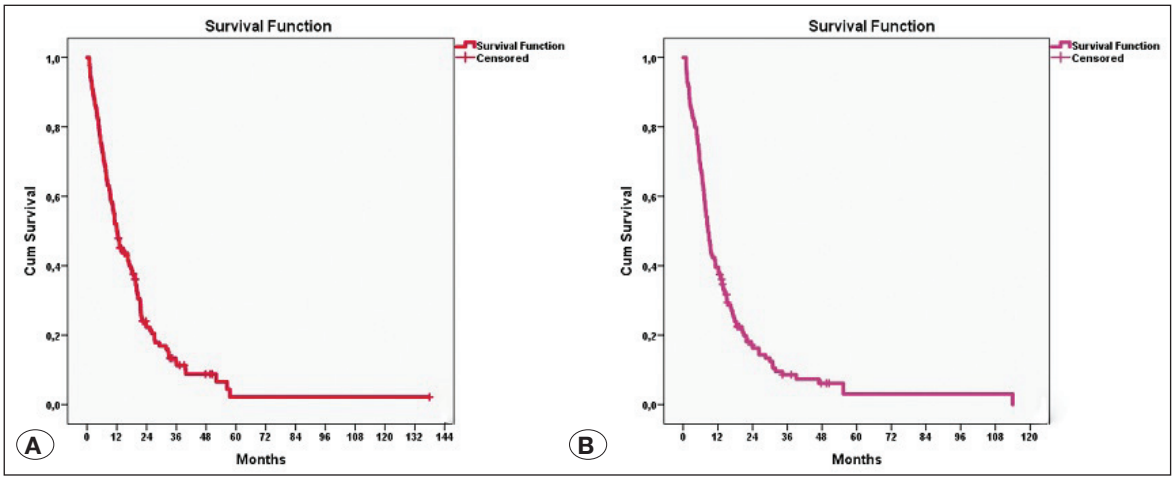

Figure 2:
A) Kaplan Meier plots of 137 GBM patients, Median Overall Survival of GBM patients,
B) progresion free survival of GBM patients.

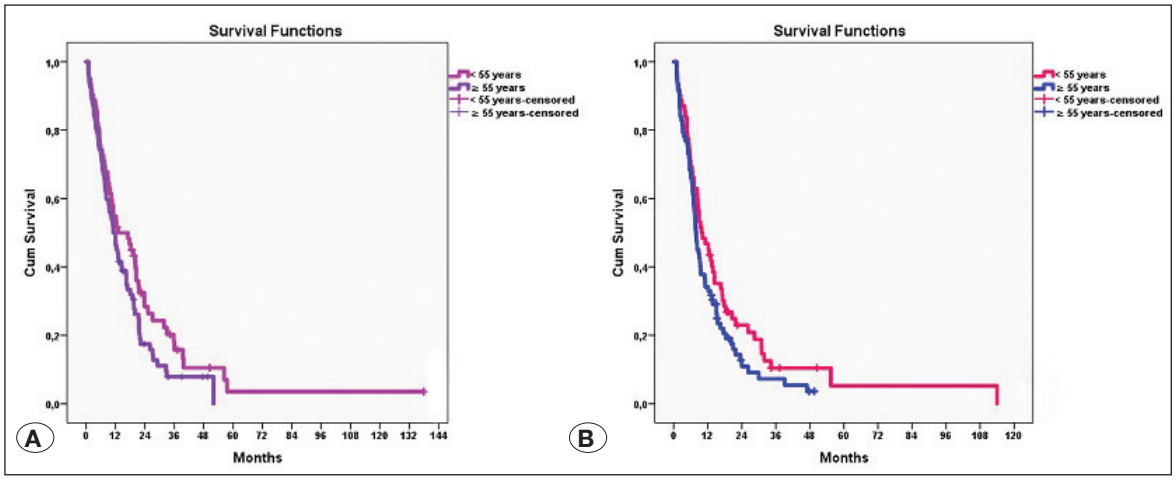

Figure 3: Kaplan Meier plots of 137 GBM patients, association between
A) overall survival and
B) progression free survival an age below and above 55 years.

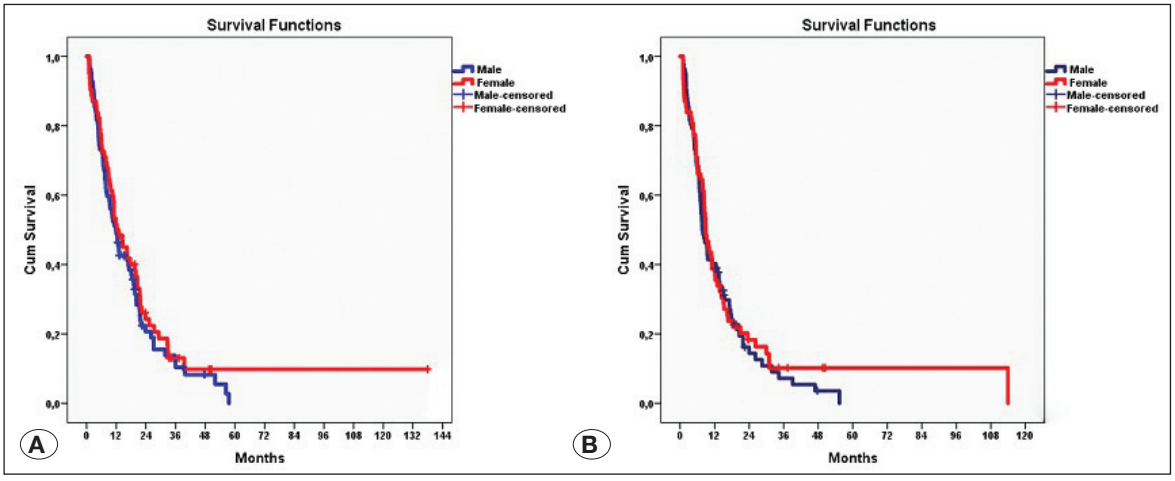

Figure 4:
A) Kaplan Meier plots of 137 GBM patients, association between male/ female and Overall Survival analysis,
B) association between male/ femal and Progression Free Survival analysis.

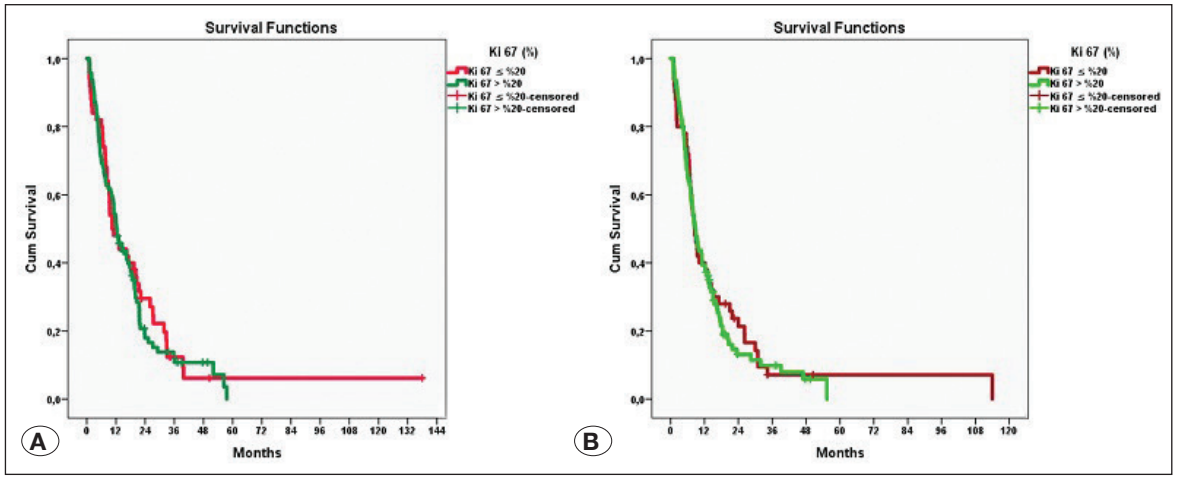

Figure 5:
A) Kaplan Meier plots of 137 GBM patients, association between Ki67 (below and above 20%) and Overall Survival analysis,
B) association between Ki67 (%) and Progression Free Survival analysis.

13.1% (n=18) had KPS levels < 70% and 86.9% (n=119) had KPS levels ≥ 70% (Table I). The median PFS and OS were significiantly longer when the KPS level was >70% at the time of diagnosis than when it was <70% (p<0.001 and p<0.001, respectively; Table III and Figure 6A, B).

IDH-1 Mutation Status and Survival

IDH-1 mutation was detected in 21 patients (15.3%; Table I) and associated with longer median PFS and OS. The median OS was 21.63 months (95% CI, 14.63–28.63) for the patients with IDH-1 mutation and 11.06 months (95% CI, 8.69–13.44) for those without IDH-1 mutation (p=0.074; Tables II and IV). The median PFS was 15.43 months for the patients with IDH-1 mutation (95% CI, 5.91–24.95) and 7.63 months (95% CI, 6.84–8.78) for those without IDH-1 mutation (p=0.043; Tables III and IV; Figure 7A, B). IDH mutation showed no statistically significant differences in OS according to the clinical, pathological, and patient characteristics.

MGMT Methylation Status and Survival

MGMT methylation (n=40; Table I) was associated with longer median PFS and OS. The median OS times were 22 months (95% CI, 19.11–24.88) and 8.83 months (95% CI, 7.07–10.58, p<0.001; Table II), and the median PFS were 15.26 months (95% CI, 11.86–18.66) and 7.03 months (95% CI, 5.8–8.26; p=0.001; Table III, Figure 8A, B) in the patients with and in those without MGMT methylation, respectively.

Treatment Modalities and Survival

One hundred twenty-eight patients were treated with chemoradiotherapy. Of the patients, 106 were treated with chemoradiotherapy and adjuvant TMZ and RT, and 62 were treated with bevacizumab+irinotecan progression after chemoradiotherapy and adjuvant TMZ therapy (Table I). The median OS 13 months (95% CI, 8.57–17.42) with RT concomitant with TMZ therapy and 1.16 months (95% CI, 0.97–1.36) without the treatment (p<0.001; Table II). The median PFS was 9.23 months (95% CI, 7.188–11.278) with RT concomittant with TMZ therapy and 1.16 months (95% CI, 0.972–1.361) without the treatment (p<0.001; Table III). The median OS times were 18.36 months (95% CI, 15.647–21.08) and 2.56 months (95% CI, 1.585–3.548; p<0.001; Table II), and the median PFS were 12.23 months (95% CI, 9.252–15.214) and 2.56 months (95% CI, 1.585–3.548; p<0.001; Table III and Figure 9A, B) with RT with concomitant and adjuvant TMZ therapies, respectively. The median OS with

Table I: Demographic Characteristics of Patients

		n	%
Age (years)	*Min-Max (Median)*	19-91 (56)	
	mean ± *Standart deviation*	56.42 ± 13.09	
	< 55 years	59	43.1
	≥ 55 years	78	56.9
Sex	**Male**	78	56.9
	Female	59	43.1
Pathologic subtype	**GBM**	137	100.0
Ki 67 (%)	*Min-Max (Median)*	10-90 (30)	
	mean ± Standart *deviation*	32.64 ± 16.56	
	Ki 67 ≤ %20	48	35.0
	Ki 67 > %20	89	65.0
KPS (%)	*Min-Max (Median)*	60-90 (70)	
	mean ± Standart *deviation*	73.50 ± 8.62	
	KPS < %70	18	13.1
	KPS ≥ %70	119	86.9
MGMT methylation	**Negative**	97	70.8
	Positive	40	29.2
IDH-1 mutation	**IDH Wild (-)**	108	78.8
	Mutant (+)	21	15.3
	NOS	8	5.8
CRT	**CRT (-)**	9	6.6
	CRT (+)	128	93.4
Adjuvant Temozolamide	**(-)**	31	22.6
	(+)	106	77.4
Bevacizumab+irinotecan combination	**No**	75	54.7
	Yes	62	45.3
Survival	**Live**	19	13.9
	Ex	118	86.1
Median Follow-up	*Min-Max (Median)*	1-137.93 (12.0)	
	mean ± Standart *deviation*	16.04 ± 16.04	
Treatment	**CRT (+)**	22	16.1
	CRT+maintenance	106	77.4
	Bevacizumab+irinotecan combination	62	45.3
Progression Status	**No**	15	10.9
	Yes	122	89.1
PFS (months)	*Min-Max (Median)*	1-113.97 (8.56)	
	mean ± Standart *deviation*	12.93 ± 13.72	

CRT: *Chemoradiotheraphy,* ***Maintenance:*** *Adjuvant temozolamide.*

bevacizumab+irinotecan therapy after RT progresssion with concomitant and adjuvant TMZ therapies, and RT concomittant with TMZ therapy was 19.36 months (95% CI, 14.803–29.931) as compared with 8.83 months (95% CI, 5.981–11.686) without the bevacizumab+irinotecan combination therapy (p=0.012; Table II and Figure 10A, B). The median PFS with bevacizumab+irinotecan therapy after RT progresssion with concomitant and adjuvant TMZ therapies and median OS with RT concomittant with TMZ therapy were both 13 months (95% CI, 8.71–17.29) as compared with 7.03 months (95% CI, 5.619–8.448) without the bevacizumab+irinotecan combination therapy (p=0.012; Table III).

Table II: Overall Survival

		n	Ex	Live	Survival rate	Median survival time	95% Confidence Interval		Log Rank Test; p
							Lower	Upper	
Age	**< 55 years**	59	50	9	15.3%	13.00 ± 3.65	5.840	20.160	0.156
	≥ 55 years	78	68	10	12.8%	11.10 ± 1.87	7.422	14.778	
Sex	**Male**	78	68	10	12.8%	11.10 ± 1.87	7.435	14.765	0.331
	Female	59	50	9	15.3%	13.00 ± 2.31	8.455	17.545	
Ki67	**Ki 67 ≤ 20%**	48	42	6	12.5%	11.06 ± 4.00	3.221	18.912	0.510
	Ki 67 > 20%	89	76	13	14.6%	12.00 ± 0.97	10.099	13.901	
KPS	**KPS < 70%**	18	18	0	0.0%	6.66 ± 0.14	6.389	6.944	**<0.001****
	KPS ≥ 70%	119	100	19	16.0%	16.36 ± 2.49	11.486	21.247	
MGMT methylation	**Negative**	97	82	15	15.5%	8.83 ± 0.89	7.079	10.588	**<0.001****
	Positive	40	36	4	10.0%	22.0 ± 1.47	19.114	24.886	
IDH mutation	**Wild (-)**	108	94	14	13.0%	11.06 ± 1.21	8.691	13.443	0.074
	Mutant (+)	21	16	5	23.8%	21.63 ± 3.57	14.632	28.634	
	NOS	8	8	0	0.0%	11.06 ± 9.75	0.000	30.192	
CRT	**CRT (-)**	9	9	0	0.0%	1.16 ± 0.09	0.972	1.361	**<0.001****
	CRT (+)	128	109	19	14.8%	13.00 ± 2.25	8.576	17.424	
Adjuvant Temozolamide	**(-)**	31	31	0	0.0%	2.56 ± 0.50	1.585	3.548	**<0.001****
	(+)	106	87	19	17.9%	18.36 ± 1.38	15.647	21.087	
Bevacizumab+irinotecan combination	**No**	75	68	7	9.3%	8.83 ± 1.45	5.981	11.686	**0.012****
	Yes	62	50	12	19.4%	19.36 ± 2.32	14.803	23.931	

CRT: *Chemoradiotheraphy,* *****:*** statistically significant.

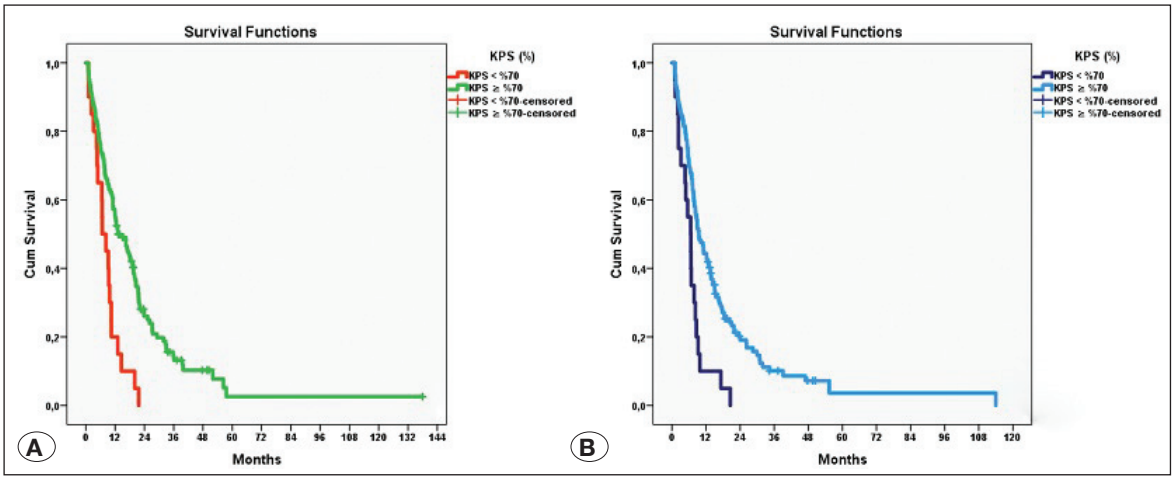

Figure 6: **A)** Kaplan Meier plots of 137 GBM patients, association between KPS (below and above 70%) and Overall Survival analysis, **B)** Association between KPS (%) and Progression Free Survival analysis.

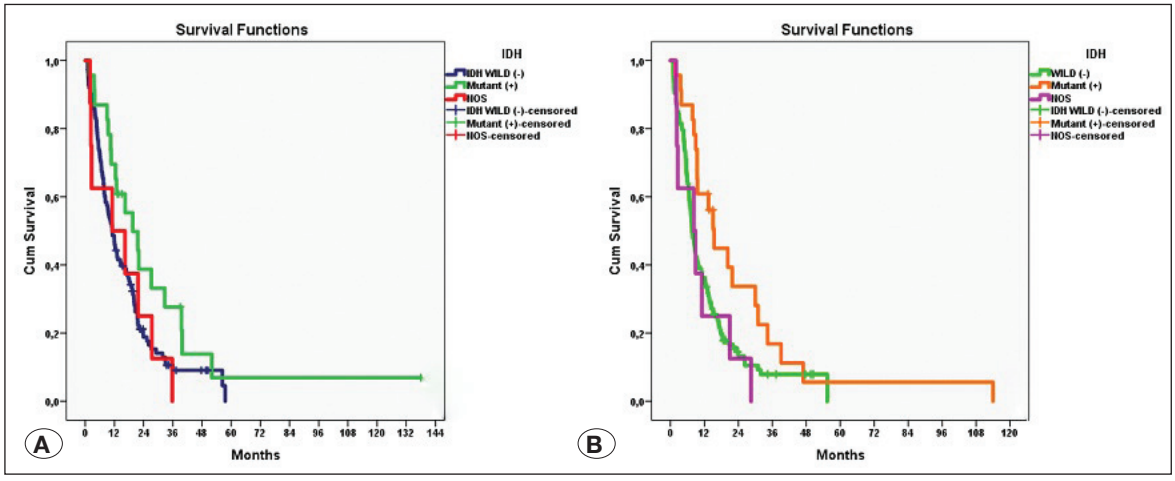

Figure 7: **A)** Kaplan Meier plots of 137 GBM patients, association IDH mutation status and overall survival analysis, **B)** association IDH mutation status and progression free survival analysis.

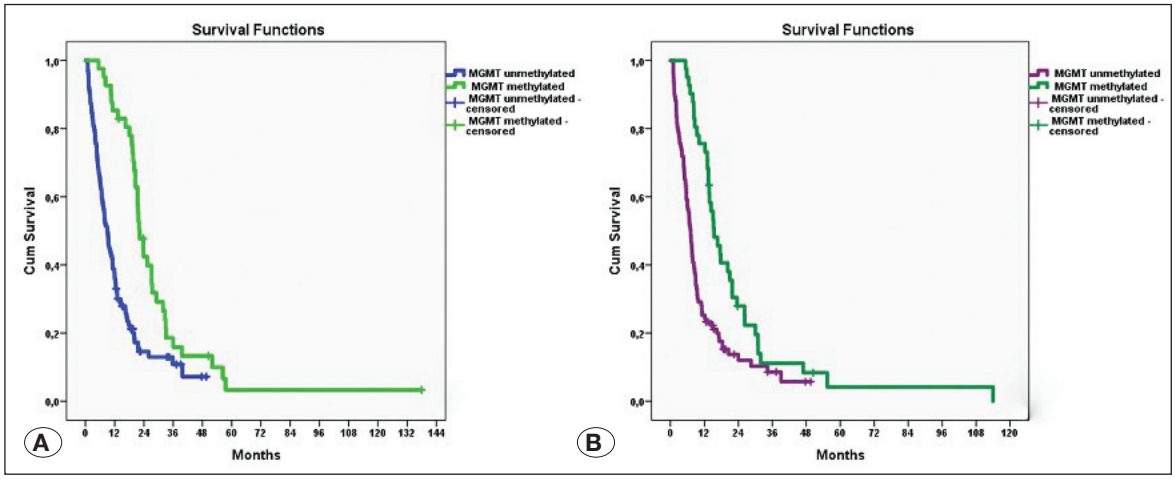

Figure 8: **A)** Kaplan Meier plots of 137 GBM patients association MGMT and overall survival analysis, **B)** association MGMT and progression free survival analysis.

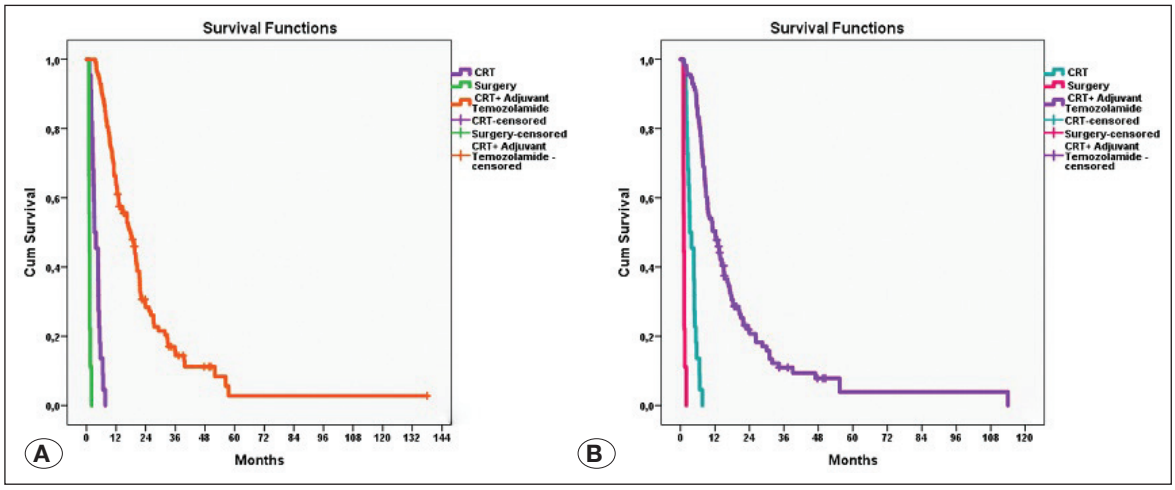

Figure 9: **A)** Kaplan Meier plots of 137 GBM patients, association treatment modalities and overall survival analysis, **B)** association treatment modalities and progression free survival analysis.

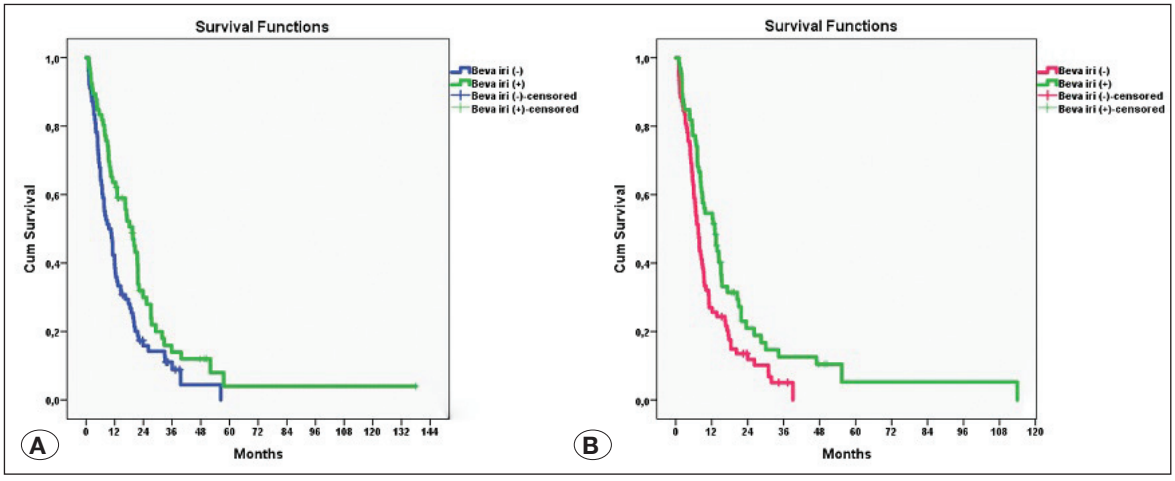

Figure 10: **A)** Kaplan Meier plots of 137 GBM patients, association Bevacizumab + irinotecan and Overall survival analysis, association Bevacizumab + irinotecan and progression free survival analysis.

Table III: Progression Free Survival

			Progression		Progression free survival		95% Confidence Interval		Log Rank Test; p
		n	Yes	No	Rate	Median time	Lower	Upper	
Age	**< 55 years**	59	52	7	11.9%	9.80 ± 2.01	5.858	13.742	0.201
	≥ 55 years	78	70	8	10.3%	7.70 ± 0.55	6.618	8.782	
Sex	**Male**	78	70	8	10.3%	7.63 ± 0.73	6.191	9.076	0.424
	Female	59	52	7	11.9%	9.23 ± 1.33	6.617	11.850	
Ki67	**Ki 67 ≤ 20%**	48	44	4	8.3%	8.56 ± 1.03	6.530	10.604	0.505
	Ki 67 > 20%	89	78	11	12.4%	8.40 ± 0.97	6.483	10.317	
KPS	**KPS < 70%**	18	18	0	0.0%	5.53 ± 1.80	1.999	9.067	**<0.001****
	KPS ≥ 70%	119	104	15	12.6%	9.56 ± 1.58	6.469	12.664	
MGMT methylation	**Negative**	97	85	12	12.4%	7.03 ± 0.62	5.805	8.262	**0.001****
	Positive	40	37	3	7,5%	15.26 ± 1.73	11.866	18.667	
IDH mutation	**Wild (-)**	108	96	12	11.1%	7.63 ± 0.58	6.484	8.782	0.043
	Mutant (+)	21	18	3	14.3%	15.43 ± 4.85	5.915	24.952	
	NOS	8	8	0	0.0%	8.23 ± 4.47	0.000	17.011	
CRT	**CRT (-)**	9	9	0	0.0%	1.16 ± 0.09	0.972	1.361	**<0.001****
	CRT (+)	128	113	15	11.7%	9.23 ± 1.04	7.188	11.278	
Adjuvant Temozolamide	**(-)**	31	31	0	0.0%	2.56 ± 0.50	1.585	3.548	**<0.001****
	(+)	106	91	15	14.2%	12.23 ± 1.52	9.252	15.214	
Bevacizumab+irinotecan combination	**No** **Yes**	75 62	70 52	5 10	6.7% 16.1%	7.03 ± 0.72 13.00 ± 2.18	5.619 8.710	8.448 17.290	**0.012****

CRT: *Chemoradiotheraphy,* *****:*** *statistically significant.*

Table IV: IDH Mutation Status

		IDH mutation			p
		IDH wild (-)	Mutant (+)	NOS	
		n (%)	n (%)	n (%)	
Age (years)	**< 55 years**	48 (44.4)	9 (42.9)	2 (25.0)	[a]0.683
	≥ 55 years	60 (55.6)	12 (57.1)	6 (75.0)	
Sex	**Male**	62 (57.4)	14 (66.7)	2 (25.0)	[a]0.129
	Female	46 (42.6)	7 (33.3)	6 (75.0)	
Ki 67 (%)	**Ki 67 ≤ 20%**	39 (36.1)	6 (28.6)	3 (37.5)	[b]0.794
	Ki 67 > 20%	69 (63.9)	15 (71.4)	5 (62.5)	
KPS (%)	**KPS < 70%**	17 (15.9)	0 (0.0)	1 (12.5)	[a]0.136
	KPS ≥ 70%	91 (84.1)	21 (100.0)	7 (87.5)	
MGMT methylation	**Negative**	80 (74.1)	11 (52.4)	6 (75.0)	[b]0.130
	Positive	28 (25.9)	10 (47.6)	2 (25.0)	
CRT	**CRT (-)**	9 (8.3)	0 (0)	0 (0)	[a]0.499
	CRT (+)	99 (91.7)	21 (100)	8 (100)	
Adjuvant temozolamide	**(-)**	26 (24.1)	2 (9.5)	3 (37.5)	[a]0.226
	(+)	82 (75.9)	19 (90.5)	5 (62.5)	
Treatment	**CRT (+)**	17 (15.7)	2 (9.5)	3 (37.5)	[a]0.268
	CRT+Maintenance	82 (75.9)	19 (90.5)	5 (62.5)	
Mortality	**Live**	14 (13.0)	5 (23.8)	0 (0)	[a]0.204
	Ex	94 (87.0)	16 (76.2)	8 (100)	
Progresion status	**No**	12 (11.1)	3 (14.3)	0 (0)	[a]0.768
	Yes	96 (88.9)	18 (85.7)	8 (100)	

[a]Fisher Freeman Halton Exact Test, [b]Pearson Ki-kare Test, ***CRT:*** *Chemoradiotheraphy,* ***Maintenance:*** *Adjuvant temozolamide.*

The Cox regression analysis was used to determine the prognostic impact of sex, MGMT methylation, IDH mutation, age at diagnosis, and preoperative KPS. The multivariate analysis revealed that methylated MGMT (p=0.004; hazard ratio [HR], 0.53; 95% CI, 0.34–0.81) and KPS levels ≥ 70% (p=0.005; HR, 0.46; 95% CI, 0.26–0.79) were independent prognostic factors.

DISCUSSION

The pathological classification of brain tumors was revised in May 2016 by the WHO to provide guidance on the driver mutations in glioma. IDH gene-mutant tumors demonstrated significiantly good clinical outcomes as compared with the wild types. IDH mutation has been recognized to be of central prognostic and biologic importance, and the diagnostic tool has been incorporated in the diagnosis since 2016 (18,30). Notably, IDH gene status has been made a major criterion for tumor classification (1,25,39). In our study, we aimed to determine the importance of IDH mutation, MGMT methylation, and cofactors such as age, sex, performance status, and treatment modalities in our patient population retrospectively.

Several retrospective trials have been conducted after the new classification has been established by the WHO. Some studies searched the prognostic significance of the parameters in their patient cohorts according to geographic distinction. For example, the high prognostic value of the new WHO histomolecular classification of gliomas was presented in the French Polo Cohort (PFS and OS; p<0.001) (35). Another study showed that the WHO reclassification had a prognostic significance in Mongolian patients, especially those with grade II tumors (24). Another study from Japan compared the prognostic value of the new WHO classification in 387 patients with glioma. The new classification more clearly shows the tumorigenesis of gliomas, highlighting the prognostic power of the classification in Japanese patients with glial tumors in this study (16). The exact prognostic importance was

demonstratedin our study, similarly in the studies from different countries. The OS rates were higher in the patients with IDH mutation, and the number of deaths was considered as the mortality rate (70%) in the patients with wild-type IDH.

IDH-mutant GBM accounts for approximately 10% of all GBMs and is often found in young patients (1,39). In our patient population, the IDH mutation rate was 16%, which correlates with the rate reported in the literature.

According to the study by DeWitt, the decreasing prevalence of IDH-1 mutation in older patients resulted in a proposal not to perform sequencing for IDH in patients with GBM aged ≥55 years (8). In our study, most of the patients with IDH mutation were between 45 and 65 years old. Before 45 years old and after 65 years old, the IDH mutation rates were low. In our patient study population, the IDH mutation rate was 3 times higher in the patients between 45 and 65 years old. Maybe the regional differences affect the IDH mutation rates in different countries in different age groups. In our study, the IDH mutation rate was higher in the patients aged ≥55 years, but the difference was not significant (p=0.540).

In 2016, the WHO published an update to the classification of CNS tumors that, for the first time, combined molecular alterations with histological findings in the diagnosis. As relatively rapid advances have transpired in understanding the impact of molecular changes for diagnosing and grading brain tumors, a new classification is planned for GBM in 2021. One major recommendation is to separate isocitrate dehydrogenase (IDH) wild-type GBMs from GBMs with IDH mutation, which tend to have a better prognosis. IDH wild-type GBMs will consist of diffuse astrocytic gliomas with the classic histological findings of microvascular proliferation or necrosis, or have the molecular features of GBM, including EGFR amplification or TERT mutation, or the combination of the gain of the entire chromosome 7 and loss of chromosome 10 (21).

Higher KPS is an important predictive factor of survival according to some studies (29). In our study, higher KPS is associated with prolonged survival. Another important aspect of good perfomance status is associated with good tolerance for therapy without toxicity.

Radiotherapy and chemotherapy (TMZ and procarbazine-lomustine-vincristine [PCV]) options that provide survival advantage in gliomas target proliferating cells nonspecifically (9,36). These treatment options are the standard of care for patients with GBM up to age 70 years (33), or suitable for elderly patients aged >70 years (11).

The feasibility of upfront testing and stratification by MGMT status has been demonstrated as the standard approach, and alternative strategies for patients with unmethylated MGMT are under development (31). MGMT methylation status is another important factor of survival. In an analysis of a subset of 206 patients in the EORTC/NCIC trial whose MGMT methylation statuses were determined retrospectively, methylation of the MGMT promotor was a major predictor of benefit from chemotherapy and a prognostic factor for prolonged survival (13). In our study, MGMT methylation was associated with polonged OS and PFS.

In the absence of effective and better treatment alternatives for GBM, combined modality with radiotherapy and TMZ therapy remains the standard of care (31). The subgroup of patients who could take advantage of the therapy has not been described yet despite the new WHO classification. In some studies, the presence of mutation plays an important role in the response to therapy that includes surgery and chemoradiotherapy (22,32). In our patient population, IDH mutation was the best predictor of the survival advantage of adjuvant chemoradiotherapy followed by TMZ therapy (p = 0.010). In the IDH mutant group in our study, the patients with wild-type IDH had a 5-month survival advantage. Unfortunately, no standard therapy has been established after the first-line therapy for recurrent tumors (32). Similary, nitrosureas such as carmustine or lomustine would be a reasonable second-line therapy (27,34,38). Although no studies that used bevacizumab for recurrent GBM have demonstrated an improvement in survival. Bevacizumab ± irinotecan showed high response rates and a steroid-sparing effect; however, the effect is frequently associated with changes in vascular permeability, but no survival advantage was demonstrated in patients with recurrent disease (10,19,31). The statistically significant survival advantage was not observed in our study, but the survival rates were 12 months higher in the patients with IDH mutation. A statistically significant survival advantage was found retrospectively in our study with all treatment lines, including RT with concomitant and adjuvant TMZ and bevacizumab+irinotecan (p=0.001, p=0.001, and p=0.008, respectively). IDH mutation status did not affect the treatment efficacies of the three treatment protocols (Table IV).

Advances in the molecular structure of gliomas provide information on why no targeted agents have been developed for gliomas for a long time (5). Therapeutic alternatives are restricted for recurrence, and treatment should be personalized because of patients' tumor burdens, functional statuses, and prior treatments differ. Ongoing research studies of novel treatment alternatives for GBM include targeted therapy and immunotherapy (23,32). In view of the importance of IDH-1/IDH-2 mutation in the development of gliomas, many IDH-1/IDH-2 inhibitors and vaccines have entered the clinical trial stage (2,7,15).

In our daily practice, the major problem is wild-type IDH, which is associated with lower patient survival rates and unsuccessful treatment modalities as compared with the IDH mutant types.

The effectiveness of surgical treatment in low-grade gliomas depends on the molecular subtype of the tumors. Patients with wild-type IDH seem to benefit from more-intensive treatments such as gross total resection, multiple resections, and combined chemo/radiotherapy. If possible, wild-type glial tumors must be treated with a more aggressive aproach in all therapy components (7).

The median OS of the patients with GBM is nearly 12 months in population-based studies in clinical trials of standard therapies (7). In our study, the patient with the longest survival (57.57 months) had wild-type IDH, a Ki67 score of 35, and a KPS of 90%, and were treated with all the three modalities

included in our study after gross total resection. This example shows that not only one factor but multiple factors, including tumor- and patient-associated moleculer and genetic factors, affect patient survival.

Our study has several limitations Several parameters limited our study owing to its retrospective nature. The method used in our study was not the only method used to determine MGMT methylation and IDH-1 mutation. For this reason, the MGMT methylation and IDH-1 mutation rates may vary when using different methods. As our study group was small, our findings should be supported by larger and more-comprehensive studies that include both primary and secondary GBM cases.

CONCLUSION

In conclusion, all the patients in the IDH mutant group had a survival advantage. The patient population had an opportunity to undergo chemoradiotherapy and chemotherapy owing to their prolonged survival. In the patients with longer survival who were treated with bevacizumab+irinotecan combination therapy, had a surival advantage, and KPS scores of >70, MGMT methylation significantly affected the PFS and OS. While the presence of IDH mutation prolonged survival, it was not statistically significant.

This study is important for elucidating the prognostic importance and survival effect of the new classification on the treatment modalities used in Turkish patients. Treatment modalities, patients' chance of response and survival with the new classification, and some clinical and pathological cofactors were analyzed in our study.

The new WHO histomolecular classification has a high prognostic value but is not predictive yet. MGMT methylation status, which is as important as IDH mutation, should be included in the new classification. New treatment modalities are needed to prolong survival.

REFERENCES

1. Beiko J, Suki D, Hess KR, Fox BD, Cheung V, Cabral M, Shonka N, Gilbert MR, Sawaya R, Prabhu SS, Weinberg J, Lang FF, Aldape KD, Sulman EP, Rao G, McCutcheon IE, Cahill DP: IDH1 mutant malignant astrocytomas are more amenable to surgical resection and have a survival benefit associated with maximal surgical resection. Neuro Oncol 16(1):81-91, 2014
2. Bhavya B, Anand CR, Madhusoodanan UK, Rajalakshmi P, Krishnakumar K, Easwer HV, Deepti AN, Gopala S: To be wild or mutant: Role of isocitrate dehydrogenase 1 (IDH1) and 2-Hydroxy glutarate (2-HG) in gliomagenesis and Treatment outcome in glioma. Cell Mol Neurobiol 40(1):53-63, 2020
3. Burgenske DM, Yang J, Decker PA, Kollmeyer TM, Kosel ML, Mladek AC, Caron AA, Vaubel RA, Gupta SK, Kitange GJ, Sicotte H, Youland RS, Remonde D, Voss JS, Barr Fritcher EG, Kolsky KL, Ida CM, Meyer FB, Lachance DH, Parney IJ, Kipp BR, Giannini C, Sulman EP, Jenkins RB, Eckel-Passow JE, Sarkaria JN: Molecular profiling of long-term IDH-wildtype glioblastoma survivors. Neuro Oncol 21(11):1458-1469, 2019
4. Carrillo J, Lai A, Nghiemphu PL, Kim HJ, Phillip HS, Kharbanda S, Pope WB: Relationship between tumor enhancement, edema, IDH1 mutational status, MGMT promoter methylation, and survival in glioblastoma. Am J Neuroradiol 33(7):1349-1355, 2012
5. Chen R, Smith-Cohn M, Cohen AL, Colman H: Glioma subclassifications and their clinical significance. Neurotherapeutics 14(2):284-297, 2017
6. Cohen MH, Shen YL, Keegan P, Pazdur R: FDA drug approval summary: Bevacizumab (Avastin) as treatment of recurrent glioblastoma multiforme. Oncologist 14:1131-1138,2009
7. Delev D, Heiland DH, Franco P, Reinacher P, Mader I, Staszewski O, Lassmann S, Grau S, Schnell O: Surgical management of lower-grade glioma in the spotlight of the 2016 WHO classification system. J Neurooncol 141(1):223-233, 2019
8. DeWitt JC, Jordan JT, Frosch MP, Samore WR, Iafrate AJ, Louis DN, Lennerz JK: Cost-effectiveness of IDH testing in diffuse gliomas according to the 2016 WHO classification of tumors of the central nervous system recommendations. Neuro-oncology 19(12):1640-1650, 2017
9. Draaisma K, Chatzipli A, Taphoorn M, Kerkhof M, Weyerbrock A, Sanson M, Hoeben A, Lukacova S, Lombardi G, Leenstra S, Hanse M, Fleischeuer R, Watts C, McAbee J, Angelopoulos N, Gorlia T, Golfinopoulos V, Kros JM, Verhaak RGW, Bours V, van den Bent MJ, McDermott U, Robe PA, French PJ: Molecular evolution of idh wild-type glioblastomas treated with standard of care affects survival and design of precision medicine trials: A report from the EORTC 1542 study. J Clin Oncol 38(1):81-99, 2020
10. Friedman HS, Prados MD, Wen PY, Mikkelsen T, Schiff D, Abrey LE, Yung WKA, Paleologos N, Nicholas MK, Jensen R, Vredenburgh J, Huang J, Zheng M, Cloughesy T: Bevacizumab alone and in combination with irinotecan in recurrent glioblastoma. J Clin Oncol 27:4733-4740, 2009
11. Gilbert MR, Wang M, Aldape KD, Stupp R, Hegi ME, Jaeckle KA, Armstrong TS, Wefel JS, Won M, Blumenthal DT, Mahajan A, Schultz CJ, Erridge S, Baumert B, Hopkins KI, Tzuk-Shina T, Brown PD, Chakravarti A, Curran Jr WJ, Mehta MP: Dose-dense temozolomide for newly diagnosed glioblastoma: A randomized phase III clinical trial. J Clin Oncol 31:4085-4091, 2013
12. Hegi ME, Diserens AC, Godard S, Dietrich PY, Regli L, Ostermann S, Otten P, Melle GV, de Tribolet N, Stupp R: Clinical trial substantiates the predictive value of O-6-methylguanine-DNA methyltransferase promoter methylation in glioblastoma patients treated with temozolomide. Clin Cancer Res 10:1871-1874, 2004
13. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M, Kros JM, Hainfellner JA, Mason W, Mariani L, Bromberg JE, Hau P, Mirimanoff RO, Cairncross JG, Janzer RC, Stupp R: MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 352(10):997-1003, 2005
14. Hodges TR, Choi BD, Bigner DD, Yan H, Sampson JH: Isocitrate dehydrogenase 1: What it means to the neurosurgeon: A review. J Neurosurg 118(6):1176-1180, 2013

15. Huang J, Yu J, Tu L, Huang N, Li H, Luo Y: Isocitrate dehydrogenase mutations in glioma: From basic discovery to therapeutics development. Front Oncol 9:506, 2019
16. Iuchi T, Sugiyama T, Ohira M, Kageyama H, Yokoi S, Sakaida T, Hasegawa Y, Setoguchi T, Itami M: Clinical significance of the 2016 WHO classification in Japanese patients with gliomas. Brain Tumor Pathol 35(2):71-80, 2018
17. Karayan-Tapon L, Quillien V, Guilhot J, Wager M, Fromont G, Saikali S, Gratas-Rabbia-Ré C: Prognostic value of O 6-methylguanine-DNA methyltransferase status in glioblastoma patients, assessed by five different methods. J Neuro-oncol 97(3):311-322, 2010
18. Kesler SR, Harrison RA, Petersen ML, Rao V, Dyson H, Alfaro-Munoz K, Weathers SP, de Groot J: Pre-surgical connectome features predict IDH status in diffuse gliomas. Oncotarget 10(60):6484-6493, 2019
19. Kreisl TN, Kim L, Moore K, Duic P, Royce C, Stroud I, Garren N, Mackey M, Butman JA, Camphausen K, Park J, Albert PS, Fine HA: Phase II trial of single-agent bevacizumab followed by bevacizumab plus irinotecan at tumor progression in recurrent glioblastoma. J Clin Oncol 27:740-745, 2009
20. Louis DN, Perry A, Reifenberger G, von Deimling A, Figarella-Branger D, Cavenee WK, Ohgaki H, Wiestler OD, Kleihues P, Ellison DW: The 2016 World Health Organization Classification of tumors of the central nervous system: A summary. Acta Neuropathol 131(6):803-820, 2016
21. Louis DN, Wesseling P, Aldape K, Brat DJ, Capper D, Cree IA, Eberhart C, Figarella-Branger D, Fouladi M, Fuller GN, Giannini C, Haberler C, Hawkins C, Komori T, Kros JM, Ng HK, Orr BA, Park SH, Paulus W, Perry A, Pietsch T, Reifenberger G, Rosenblum M, Rous B, Sahm F, Sarkar C, Solomon DA, Tabori U, van den Bent MJ, von Deimling A, Weller M, White VA, Ellison DW: IMPACT-NOW update 6: new entity and diagnostic principle recommendations of the cIMPACT-Utrecht meeting on future CNS tumor classification and grading. Brain Pathology 30(4):844-856, 2020
22. Michaelsen SR, Christensen IJ, Grunnet K, Stockhausen MT, Broholm H, Kosteljanetz M, Poulsen HS: Clinical variables serve as prognostic factors in a model for survival from glioblastoma multiforme: An observational study of a cohort of consecutive non-selected patients from a single institution. BMC Cancer 13:402, 2013
23. Nam JY, De Groot JF: Treatment of glioblastoma. J Oncol Practice 13(10):629-638, 2017
24. Ochirjav E, Enkhbat B, Baldandorj T, Choe G: Reclassification of mongolian diffuse gliomas according to the revised 2016 World Health Organization central nervous system tumor classification. J Pathol Transl Med 53(5):298-307, 2019
25. Okita Y, Narita Y, Miyakita Y, Ohno M, Matsushita Y, Fukushima S, Sumi M, Ichimura K, Kayama T, Shibui S: IDH1/2 mutation is a prognostic marker for survival and predicts response to chemotherapy for grade II gliomas concomitantly treated with radiation therapy. Int J Oncol 41(4):1325-1336, 2012
26. Poulsen HS, Urup T, Michaelsen SR, Staberg M, Villingshøj M, Lassen U: The impact of bevacizumab treatment on survival and quality of life in newly diagnosed glioblastoma patients. Cancer Manag Res 6:373-387,2014
27. Reithmeier T, Graf E, Piroth T, Trippel M, Pinsker MO, Nikkhah G: BCNU for recurrent glioblastoma multiforme: Efficacy, toxicity and prognostic factors. BMC Cancer 10:30, 2010
28. Salimi A, Bahiraei T, Ahdeno S, Vatanpour S, Pourahmad J: Evaluation of cytotoxic activity of betanin against U87MG human glioma cells and normal human lymphocytes and its anticancer potential through mitochondrial pathway. Nutrition and Cancer 73(3):450-459, 2020
29. Schwartz C, Romagna A, Stefanits H, Zimmermann G, Ladisich B, Geiger P, Rechberger J, Winkler S, Weiss L, Fastner G, Trinka E, Weis S, Spiegl-Kreinecker S, Steinbacher J, McCoy M, Johannes T, Gruber A, Jahromi BR, Niemelä M, Winkler PA, Thon N: Risks and benefits of glioblastoma resection in older adults: A retrospective austrian multicenter study. World Neurosurg 133:e583-e591, 2020
30. Shirahata M, Ono T, Stichel D, Schrimpf D, Reuss DE, Sahm F, Koelsche C, Wefers A, Reinhardt A, Huang K, Sievers P, Shimizu H, Nanjo H, Kobayashi Y, Miyake Y, Suzuki T, Adachi JI, Mishima K, Sasaki A, Nishikawa R, Bewerunge-Hudler M, Ryzhova M, Absalyamova O, Golanov A, Sinn P, Platten M, Jungk C, Winkler F, Wick A, Hänggi D, Unterberg A, Pfister SM, Jones DTW, van den Bent M, Hegi M, French P, Baumert BG, Stupp R, Gorlia T, Weller M, Capper D, Korshunov A, Herold-Mende C, Wick W, Louis DN, von Deimling A: Novel, improved grading system(s) for IDH-mutant astrocytic gliomas. Acta Neuropathol 136(1):153-166, 2018
31. Stupp R, Brada M, van den Bent MJ, Tonn JC & Pentheroudakis GESMO: High-grade glioma: ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Annals of Oncology 25:iii93-iii101, 2014
32. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJB, Janzer RC, Ludwin SK, Allgeier A, Fisher B, Belanger K, Hau P, Brandes AA, Gijtenbeek J, Marosi C, Vecht CJ, Mokhtari K, Wesseling P, Villa S, Eisenhauer E, Gorlia T, Weller M, Lacombe D, Cairncross JG, Mirimanoff RO, European Organisation for Research and Treatment of Cancer Brain Tumour and Radiation Oncology Groups; National Cancer Institute of Canada Clinical Trials Group: Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 10:459-466, 2009
33. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJB, Belanger K, Brandes AA, Marosi C, Bogdahn U, Curschmann J, Janzer RC, Ludwin SK, Gorlia T, Allgeier A, Lacombe D, Cairncross JG, Eisenhauer E, Mirimanoff RO, European Organisation for Research and Treatment of Cancer Brain Tumor and Radiotherapy Groups; National Cancer Institute of Canada Clinical Trials Group: Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. N Engl J Med 352:987-996, 2005
34. Taal W, Oosterkamp HM, Walenkamp AME, Dubbink HJ, Beerepoot LV, Hanse MCJ, Buter J, Honkoop AH, Boerman D, De Vos FYFL, Dinjens WNM, Enting RH, Taphoorn MJB, van den Berkmortel FWPJ, Jansen RLH, Brandsma D, Bromberg JE, van Heuvel I, Vernhout R, van der Holt B, van den Bent MJ: 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(9):943-953, 2014

35. Tabouret E, Nguyen AT, Dehais C, Carpentier C, Ducray F, Idbaih A, Mokhtari K, Jouvet A, Uro-Coste E, Colin C, Chinot O, Loiseau H, Moyal E, Maurage CA, Polivka M, Lechapt-Zalcman E, Desenclos C, Meyronet D, Delattre JY, Figarella-Branger D, for POLA Network: Prognostic impact of the 2016 WHO classification of diffuse gliomas in the French POLA cohort. Acta Neuropathol 132(4):625-634, 2016
36. Tesileanu CMS, Dirven L, Wijnenga MMJ, Koekkoek JAF, Vincent AJPE, Dubbink HJ, Atmodimedjo PN, Kros JM, van Duinen SG, Smits M, Taphoorn MJB, French PJ, van den Bent MJ: Survival of diffuse astrocytic glioma, IDH1/2-wildtype, with molecular features of glioblastoma, WHO grade IV: A confirmation of the cIMPACT-NOW criteria. Neuro Oncol 22(4):515-523, 2020
37. Waker CA, Lober RM: Brain tumors of glial origin. Adv Exp Med Biol 1190:281-297, 2019
38. Wick W, Puduvalli VK, Chamberlain MC, van den Bent MJ, Carpentier AF, Cher LM, Mason W, Weller M, Hong S, Musib L, Liepa AM, Thornton DE, Fine HA: Phase III study of enzastaurin compared with lomustine in the treatment of recurrent intracranial glioblastoma. J Clin Oncol 28(7):1168-1174, 2010
39. Wu F, Chai RC, Wang Z, Liu YQ, Zhao Z, Li GZ, Jiang HY: Molecular classification of IDH-mutant glioblastomas based on gene expression profiles. Carcinogenesis 40(7):853-860, 2019