Outcome of Combined Temozolomide/CCNU versus Temozolomide (Standard or Extended) For Patients with Methylated MGMT Glioblastoma

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Abstract: Glioblastoma treatment is a real challenge despite better understanding of molecular characterization of gliomas. More effective treatment protocols are urgently required. The current study compared the efficacy of combined Temozolomide/CCNU versus temozolomide (standard or extended) for patients with methylated O6-methylguanine-DNA-methyltransferase (MGMT) Glioblastoma.

Method: The current trial is a prospective, randomized, open-label study including ninety participants initially diagnosed with glioblastoma isocitrate dehydrogenase (IDH) wildtype, methylated MGMT promoter. The Participants were randomly allocated (1:1:1) to concomitant and adjuvant temozolomide/CCNU combined therapy, standard temozolomide or temozolomide extended therapy. The main end point was overall survival. Other endpoints were progression-free survival and toxicity.

Results: In the current study, the median overall survival was significantly higher in temozolomide/CCNU group (43 months) and extended temozolomide group (40 months) compared to standard temozolomide group (30 months). The median progression free survival was longer with temozolomide/CCNU and extended temozolomide compared to standard temozolomide without significant correlation (95% CI 0.815-1.496; *p*=0.055).

Conclusion: Multivariate analysis revealed that Eastern Cooperative Oncology Group (ECOG) scale ≤1 continued as a significant prognostic variant with overall survival in different treatment groups *Conclusion*: The effectiveness and safety of combined CCNU/ temozolomide and extended adjuvant temozolomide should be considered for treatment of recently diagnosed IDH-wildtype glioblastoma with MGMT methylated.

Keywords: Glioblastoma, Temozolomide, CCNU, Extended, MGMT.

INTRODUCTION

Glioblastoma is the most frequently occurring central nervous system (CNS) malignant tumor in adults. Tumors formerly classified as glioblastoma have now two distinct diagnoses based on IDH mutation status. The World Health Organization (WHO) Classification of CNS Tumors categorized glioblastoma as IDH-wildtype glioblastoma [1].

Radiotherapy and temozolomide (TMZ) are fundamental initial therapy for glioblastoma after resection. Patient age and *MGMT* promotor methylation are crucial prognostic and predictive factors that guide treatment strategy [2]. The European Organization for Research and Treatment of Cancer/National Cancer Institute of Canada (EORTC/ NCIC) proved that temozolomide (concomitant and adjuvant) was associated with a remarkable clinical improvement, significant survival advantage and tolerable toxicity [3].

The addition of temozolomide significantly improved the 2-year overall survival compared with radiotherapy alone for patients with *MGMT* methylation in the EORTC/NCIC trial (46 versus 23%; median overall survival 21.7 versus 15.3 months, HR 0.45, 95% CI 0.32-0.61) [3,4].

Appropriate systemic therapy for patients with glioblastoma depends on adequate molecular characterization. More effective therapies are required for better prognosis. The combination of temozolomide/ lomustine with radiation therapy, use of extended adjuvant temozolomide, testing immunotherapy or vaccine-based therapies may be beneficial [5,6].

A more intensive therapeutic regimen for physically fit, younger patients with MGMT-methylated glioblastoma involves the combination of temozolomide/ lomustine with radiotherapy that may optimize the overall survival compared with standard temozolomide therapy [7].

The effectiveness of extended temozolomide for glioblastoma patients with MGMT methylated is

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promising. Two Australian studies are ongoing to evaluate the safety and effectiveness of extended temozolomide for glioblastoma patients considering the stratification of MGMT status [8].

The current study evaluates the oncological outcome of combined CCNU/TMZ compared to standard or extended TMZ for patients *with* MGMT-methylated tumors.

PATIENTS AND METHODS

Our trial is a prospective, open-label study. Ninety participants were recruited in Clinical Oncology Department-Tanta University Hospitals through the period from January 2020 to July 2023. The Research Ethics Committee of Faculty of Medicine - Tanta University approved the study (Approval code number: 36264PR272/7/23). A written informed consent was gathered from all participants.

The patients were randomly allocated (1:1:1) to either temozolomide /CCNU, temozolomide standard therapy or temozolomide extended therapy.

Inclusion and Exclusion Criteria

The current study included ninety participants recently diagnosed with MGMT methylated glioblastoma (IDH-wildtype), aged 18–70 years, ECOG scale ≤1 and had adequate hematological and biochemical profile.

Exclusion criteria included IDH-mutant astrocytoma, GBM with unmethylated MGMT, previous chemotherapy or radiotherapy.

Pre-Treatment Evaluation

The participants were assessed by careful history taking and complete neurological examination. Surgical intervention was done at Neurosurgery Department, Al-Azhar University Hospitals and cases were referred back to Clinical Oncology Department-Tanta University Hospitals for postoperative treatment and follow up. Baseline contrasted magnetic resonance imaging (MRI) brain was done after surgery/biopsy and every 12 weeks during the study. Baseline hematological and biochemical profile were obtained for all patients and at the beginning of each course.

Histopathological Examination

Glioblastoma cases were identified according to the 2021 WHO Classification of Tumors of the CNS (WHO CNS5) guidelines, as an adult high grade (CNS WHO grade 4), diffuse astrocytic tumor that is IDH-wildtype and H3-wildtype, and demonstrate one of the following histopathological or molecular features: necrosis (palisading or not), microvascular proliferation, EGFR gene amplification, TERT promotor mutation, +7/-10 chromosome copy-number changes [9]. Haematoxylin and eosin (H&E)-stained sections were examined to establish the primary histopathological diagnosis (adult diffuse high grade astrocytic tumour), followed by selection of adequate paraffin blocks for immunohistochemical analysis, establishing the final diagnosis of Glioblastoma IDH-wildtype, CNS WHO grade 4, methylated MGMT promoter. Histopathological examination and immunohistochemical analysis was performed at Pathology Department, faculty of medicine, Tanta University.

Immunohistochemical Analysis

Immunohistochemical assessment was performed on formalin-fixed paraffin-embedded (FFPE)-selected blocks from the tumor. The (FFPE) blocks were sectioned on positively charged slides (5 µm thick), then were dried at 37°C for 30 min. The slides were placed in Dako PT Link unit for deparaffinization and antigen retrieval. EnVisionTM FLEX Target Retrieval Solution with a high pH was used for 20 minutes at 97°C. Immunohistochemistry was performed using Dako Auto stainer Link 48. Slides were immersed for 10 minutes in Peroxidase-Blocking Reagent, incubated with primary antibodies utilized in the current study. Subsequently, the slides were treated with horseradish peroxidase polymer reagent for 20 minutes and with diaminobenzidine chromogen for 10 minutes. Finally, the slides were counterstained with hematoxylin. The primary

antibodies utilized in this study were glial fibrillary acidic protein (GFAP) (rabbit polyclonal anti-GFAP IgG, clone A14673, Dilution 1:100, ABclonal, Massachusetts, United States), IDH1 (rabbit monoclonal anti-R132H-IDH1 IgG, clone ARC54138, Dilution 1:100, ABclonal, Massachusetts, United States), and MGMT (mouse Anti-MGMT IgG antibody, clone MT3.1, Dilution 1:50, Medaysis, California, United States).

Evaluation of Immunohistochemical Staining

Expression of GFAP:

Tumor cells in all cases showed positive cytoplasmic staining for GFAP, confirming the glial (astrocytic) origin of these tumors [10].

Expression of IDH

The granular cytoplasmic staining of tumor cells that is typically seen in IDH- mutant glial tumours was absent in all of the cases included in the current study which were primarily diagnosed as adult diffuse high grade astrocytoma [11]. Lack of IDH staining in these tumours was consistent with the diagnosis of Glioblastoma IDH-wildtype, CNS WHO grade 4.

Expression of MGMT

MGMT expression in tumor cells (nuclear staining) was evaluated and scored via a 3-tiered scale (3: > 50% positive tumor cells, 2: 10% to 50% positive tumor cells, 1: negative or limited to <10% positive tumor cells). All of the cases included in the current study showed negative staining to MGMT or limited staining to less than 10% positive tumor cells (score 1), reflecting *MGMT* promoter methylation [12].

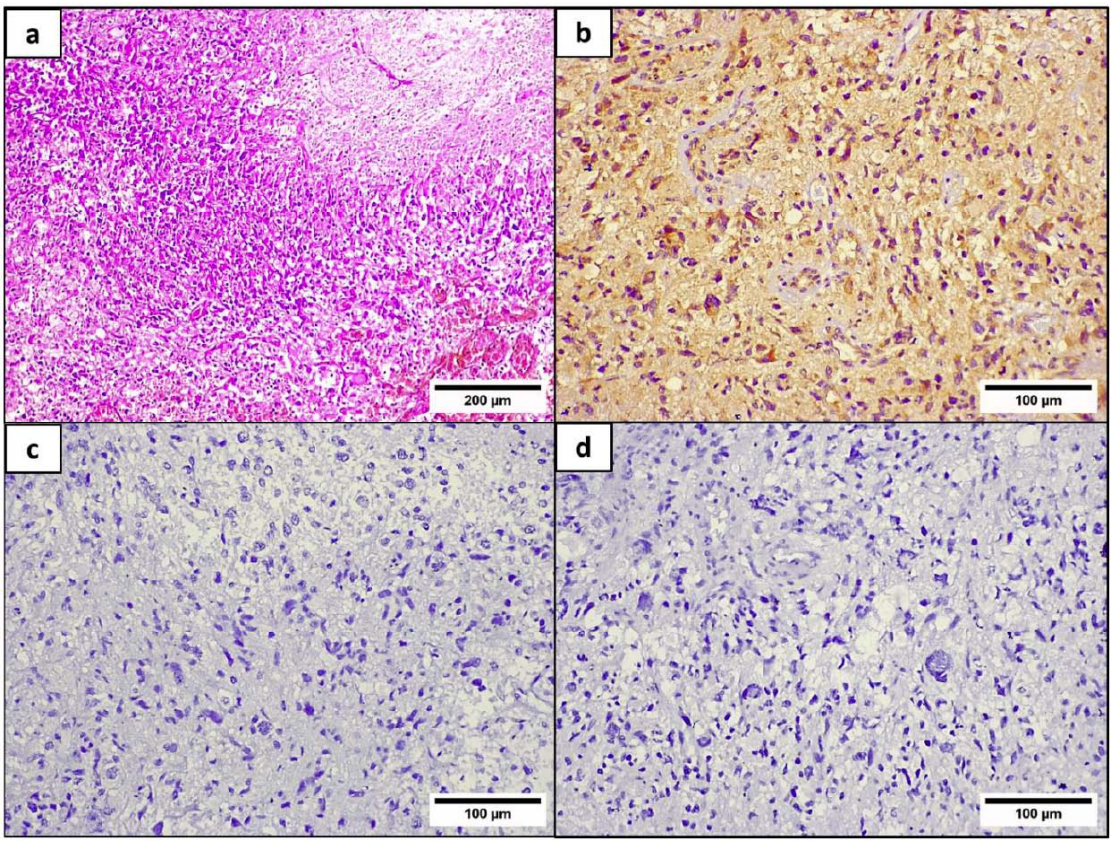
Treatment Radiotherapy

All Patients received involved-field radiotherapy (60 Gy/30 fractions/2Gy per fraction) 3-4 weeks postoperatively. Two radiotherapy volumes were used, planned target volume1 (PTV1) to 46 Gy and PTV2 to 60 Gy. PTV1, comprising the contrast enhancing tumor and surrounding FLAIR abnormality with 2 cm anatomically constrained expansion, received 46 Gy (23 fractions). The PTV2 (boost volume) involved enhancing tumor alone with a 2 cm anatomically constrained expansion, receiving 14 Gy (7 fractions) to complete 60 Gy (total dose).

Intensity-modulated RT (IMRT) was used for radiotherapy planning and beam delivery [9,10].

Concomitant and Adjuvant Therapy

- In temozolomide/CCNU arm, patients received 6–week cycle of temozolomide/CCNU orally (Day 1: CCNU 100 mg/m² and Days 2–6: temozolomide 100 mg/m²) to complete a total of 6 courses. Concomitant temozolomide was allowed**.**
- In temozolomide standard arm**,** patients received temozolomide orally (75 mg/m²) concomitant daily with radiotherapy then adjuvant



Photograph 1: A case of Glioblastoma IDH-wildtype, CNS WHO grade 4, methylated MGMT promoter: high grade tumor that shows typical palisading necrosis and microvascular proliferation (**a**) and expressing cytoplasmic staining for GFAP, confirming the glial (astrocytic) origin (**b**) IDH staining is absent in tumour cells (wild-type) (**c**) All tumor cells are lacking the nuclear expression of MGMT (score 1), denoting *MGMT* promoter methylation (**d**) (**a**: H&E, x100, scale bar 200 µm; **b**: GFAP IHC, x200, scale bar 100 µm; **c**: IDH IHC, x200, scale bar 100 µm; **d**: MGMT IHC, x200, scale bar 100 µm).

temozolomide for 6 cycles (150–200 mg/m² daily/ *5 days/ 4 weeks*).

- In temozolomide extended arm: patients received temozolomide orally (75 mg/m²) concomitant daily with radiotherapy then adjuvant temozolomide for 12 cycles (150–200 mg/m² daily/5 days/4 weeks).

Follow Up

Complete neurological examination was done at the beginning of each course, and every 3 months until the end of the study. The Response Assessment in Neuro-Oncology (RANO) criteria were considered to assess the response, using MRI images every 3 months. Toxicity was assessed based on CTCAE (Version 5.0). The least follow-up period was 6 months.

Clinical End Points

The primary endpoint was overall survival (OS) calculated from the day of randomization to death or last follow up. Secondary endpoints included progression free survival (PFS) calculated from day of randomization till the occurrence of progressive contrast-enhancing lesions according to RANO criteria. Additional secondary endpoints included toxicity based on CTCAE version 5.0.

Statistical Analysis

IBM SPSS software (Statistical Package for the Social Sciences, version 23.0) was employed to analyze data. Clinicopathological criteria, treatment protocols and adverse events were calculated by the chi-square test for categorical variables.

The survival outcomes were assessed by Kaplan Meier technique and log-rank test. Cox proportional was considered for univariate and multivariate analysis. P value$<$ 0.05 is significant. The power of the trial was assumed to be 80% and we assumed that 5% would discontinue treatment throughout the trial.

RESULTS

Patients' Characteristics

The current study included 90 cases initially diagnosed as IDH-wild type glioblastoma, MGMT methylated. The Participants were randomly assigned (1:1:1) to temozolomide /CCNU, standard temozolomide or temozolomide extended therapy.

Table 1: Patients' Characteristics

	Combined Temozolomide +CCNU		Standard Temozolomide		Extended Temozolomide		Total		*P* value
	no	%	no	%	no	%	no	%	
Median age (range): 50 (26-66)									
>50	15	50	11	36.7	20	66.7	42	46.7	0.066
≤50	15	50	19	63.3	10	33.3	48	53.3	
Sex									
Male	18	60	21	70	21	70	60	66.7	0.638
Female	12	40	9	30	9	30	30	33.3	
ECOG scale									
0-1	24	80	21	70	27	90	72	80	0.153
2	6	20	9	30	3	10	18	20	
Smoking status									
No	21	70	12	40	15	50	33	36.7	0.168
Yes	6	20	15	50	12	40	48	53.3	
Ex-smoker	3	10	3	10	3	10	9	10	
Family history									
No	27	90	24	80	27	90	78	86.7	0.421
Yes	3	10	6	20	3	10	12	13.3	
Extent of resection									
GTR	3	30	1	3.3	6	20	10	11.2	0.308
STR	25	83.3	28	93.3	23	76.7	76	84.4	
Biopsy	2	6.7	1	3.3	1	3.3	4	4.4	

Abbreviations: ECOG, Eastern Cooperative Oncology Group; GTR, gross total resection; STR, subtotal resection.

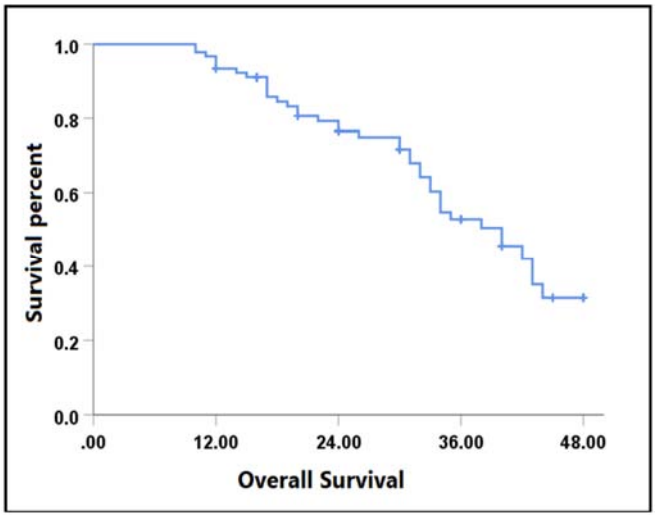


Figure 1: Kaplan-Meier curve of OS for 90 patients with MGMT-methylated glioblastoma.

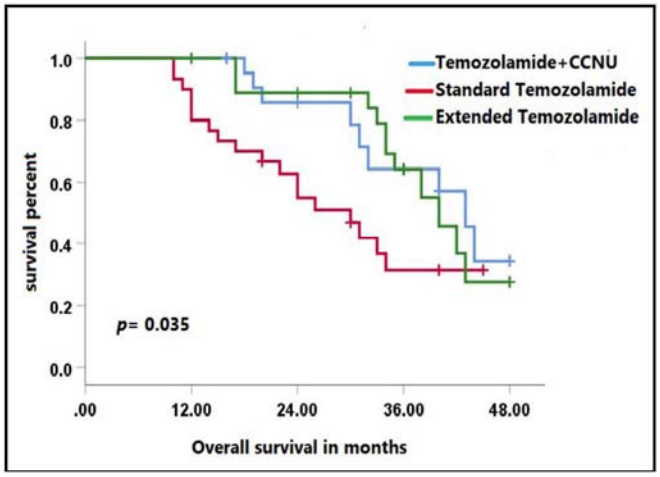


Figure 2: Kaplan-Meier curves of OS for patients with MGMT-methylated glioblastoma in three treatment groups.

The median age was 50 (26-66) years old, 48 (53.3%) patients were younger than 50 years and sixty (66.7%) participants are male patients. Most patients (80%) had ECOG scale 0-1 upon diagnosis. A family history of glioblastoma or colorectal cancer was reported in twelve (13.3%) patients.

Ten patients underwent gross total resection (GTR) at initial diagnosis; most of participants (84.4%) had subtotal resection (STR). The period of follow-up extended from 6 to 48 months. The median follow-up was 25 months. Clinicopathological features were collected in Table **1**.

All participants completed radiotherapy course to a total dose of 60 Gy. Twenty-four (80%) patients in temozolomide /CCNU arm and 90% in extended temozolomide arm completed all chemotherapy courses. All patients received six courses temozolomide in standard arm.

Overall Survival

For the entire study, the 2-year OS rate was 76.5 % (Figure **1**). The median OS was significantly better in temozolomide/CCNU and extended temozolomide arms compared to standard temozolomide arm (Figure **2**). The median OS was 43 months in temozolomide/CCNU arm and 40 months in extended temozolomide arm compared to 30 months in standard temozolomide (95% CI (1.690-2.460); *p*=0.035). Overall survival rate was significantly better in patients with ECOG scale ≤1 (95% CI 0.157:0.896; *p*= 0.014) (Table **2**).

Multivariate analysis revealed that ECOG scale ≤1 continued as significant prognostic factor with overall survival in different treatment groups [HR =0.373; 95% CI (0.156:0.893); P=0 .027] (Table **2**).

Progression Free Survival

The PFS rate was 27.8% over two years for the entire study (Figure **3**). The median PFS was 20

Table 2: Univariate and Multivariate Analysis for Overall Survival

Variables	Univariate analysis		Multivariate analysis	
	HR (95%CI)	P	HR (95%CI)	*P*
Age	0.576 (0.295: 1.122)	0.096	-	
Sex	1.95 (0.912: 4.187)	0.076		
ECOG scale	0.375 (0.157:0.896)	0.014*	0.373(0.156:0.893)	0 .027*
Smoking	0.713 (0.397: 1.280)	0.253	-	
Family history	2.135 (0.872: 5.229)	0.125	-	
Extent of Resection	0.489(0.212-1.123)	0.240	-	
Treatment modalities	1.004 (1.690-2.460)	0.035*	1.038(0.725:1.485)	0.839

Abbreviations: ECOG, Eastern Cooperative Oncology Group.
*Significant.

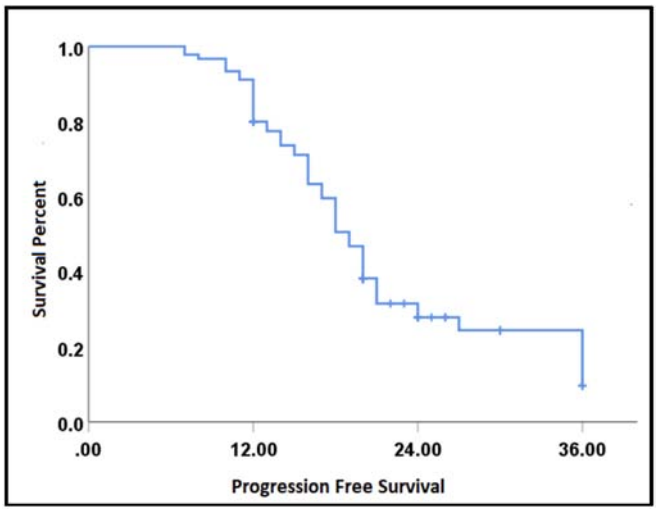


Figure 3: Kaplan-Meier curve of PFS for 90 patients with MGMT-methylated glioblastoma.

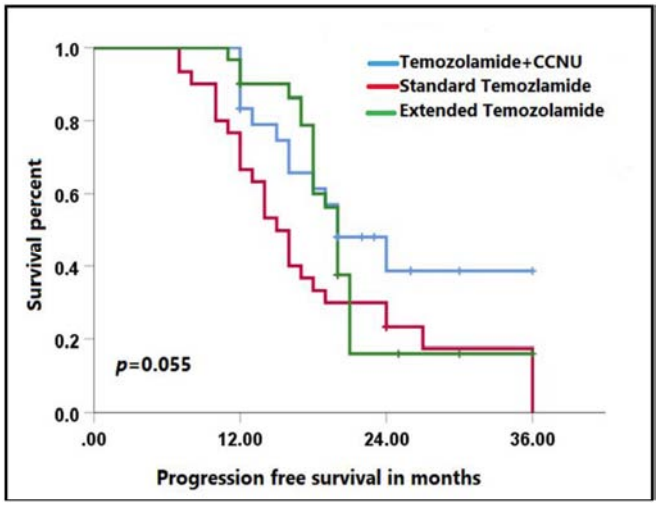


Figure 4: Kaplan-Meier curves of PFS for patients with MGMT-methylated glioblastoma in three treatment groups.

months in temozolomide/CCNU arm and extended temozolomide arm compared to 15 months in standard temozolomide with no significant correlation (95% CI 0.815-1.496; *p*=0.055) (Figure **4**). The PFS was significantly higher in patients with ECOG scale ≤1 (HR =2.251; *P*= 0.004) (Table **3**).

Toxicity

Table **4** included treatment related toxicity in the current study according to CTCAE (Version 5). There was no treatment-related mortality that occurred in our study. In the whole study, we reported grade 3-4 hematological adverse events in 38 patients (42.2%) patients while 14 patients (15.6%) had Grade 3-4 non-hematologic toxicity.

Grade 3-4 hematologic toxicity was higher with combined TMZ/CCNU and extended TMZ compared to standard temozolomide but without significant difference [14 (46.7%) patients in combined arm, 13 (43.3%) patients in extended arm versus 11 (36.7%) in the standard arm]. Lymphopenia and thrombo-cytopenia were the most common hematological toxicities reported %7.8 and 23% respectively.

Five (16.7%) patients in combined arm, and six (20%) in extended arm developed grade 3 non-hematologic toxicity. Nausea and constipation were the most common observed events. Nausea was higher in extended TMZ arm (6.7%) while constipation was higher in combined arm without significant difference. Elevated transaminase was higher in both extended and combined arms. None of the participants developed grade 4 nonhematological toxicities.

DISCUSSION

Glioblastoma treatment is a real challenge depending mainly on adequate molecular features.

Table 3: Univariate and Multivariate Analysis for Progression Free Survival

Variables	Univariate analysis		Multivariate analysis	
	HR (95%CI)	P	HR (95%CI)	*P*
Age	0.770(0.455-1.303)	0.307	-	
Sex	0.847(0.498-1.441	0.536	-	
ECOG scale	2.251(1.246-4.067	0.004*	-	
Smoking	0.886(0.577-1.360	0.096	-	
Family history	1.847(0.956-3.566	0.052	-	
Extent of Resection	0.548(0.280-1.072	0.176	-	
Treatment modalities	1.104(0.815-1.496	0.055	-	

Abbreviations: ECOG, Eastern Cooperative Oncology Group.
*significant.

Table 4: Toxicity According to CTCAE—Version 5

Grade 3-4 toxicity	Combined		Standard		Extended		Total		*p*
	no	%	no	%	no	%	no	%	
Hematological events	14	46.7	11	36.7	13	43.3	38	42.2	0.727
Neutropenia	3	10	1	3.3	1	3.3	5	5.6	0.429
Lymphopenia	2	6.7	2	6.7	3	10	7	7.8	0.856
Thrombocytopenia	8	26.7	6	20	7	23.3	21	23.3	0.830
Anemia	1	3.3	2	6.7	2	6.7	5	5.6	0.809
Nonhematological events	5	16.7	3	10	6	20	14	15.6	0.553
Nausea	2	6.7%	1	3.3	2	6.7	5	5.6	0.809
Vomiting	1	3.3%	0	0	1	3.3	2	2.2	0.600
Constipation	2	6.7	1	3.3	1	3.3	4	4.4	0.770
Diarrhea	-	-	-	-	-	-	-	-	-
Elevated transaminases	1	3.3	0	0	2	6.7	3	3.3	0.355

More effective therapies are required for better prognosis. Combining CCNU/temozolomide and extended temozolomide are considerable treatment options for glioblastoma with MGMT methylated promotor**.**

The median OS in our study was significantly higher in temozolomide/CCNU group (43 months) and extended temozolomide group (40 months) compared to standard temozolomide group (30 months) [hazard ratio (HR)=1.004; *p*=0.035]. The median OS in our trial exceeded the survival times with standard temozolomide course in the EORTC/NCIC trial (median OS=21.7 months), the CENTRIC study (median OS =26.3 months) and the CheckMate 548 trial (median OS =32.1 months) [3, 15,16].

The Stupp trial and CENTRIC trial had selectively inclusion criteria (e.g., younger patients, excellent performance status). Our single-center cohort might naturally perform differently due to these baseline variations [3, 15].

However, the median OS for our patients was shorter than those in CeTeG trial (48.1 months). The difference in median OS in our study versus the CeTeG trial could be that our study exclusively included IDH-wildtype glioblastoma patients while 23% of participants in the CeTeG trial had either unknown IDH status or IDH-mutant [7].

In the current study, the median PFS in temozolomide/CCNU and extended temozolomide groups was better than the experimental arm in CeTeG trial (20 months versus 16.7 months). It is probable that glioblastoma with methylated MGMT promotor continues to achieve response with extended TMZ (> 6 adjuvant cycles) and we randomly assigned glioblastoma participants only with methylation of the MGMT.

The efficacy of extended temozolomide for patients with glioblastoma is a matter of debate [17,18]. A meta-analysis involving seven comparative studies evaluated the effect of extended TMZ in adjuvant setting. The results revealed significant improvement in PFS (3.8 months, P < 0.001) and OS (2.3 months, P =0.18) with TMZ >6 adjuvant cycles compared to standard TMZ, but none of the included RCTs used MGMT methylation to enforce the outcome [19].

The current study evaluated the efficacy for extended TMZ in patients with glioblastoma. Extended TMZ achieved improvement in OS and PFS in GBM with MGMT methylation (median OS was 40 months and PFS was 20 months), in agreement with Wang study that reported median OS of 36 months and the median PFS was 22 months with extended temozolomide (12 cycles) for high grade glioma [20]. Gramatzki *et al*, reported nearly similar results for patients with GBM received extended temozolomide course (>6 cycles) and achieved PFS of 20.4 months [21].

The combined and extended treatment arms in addition to ECOG scale ≤1 continued as significant prognostic factor with overall survival in our cohort. Gross total resection is highly prognostic in glioblastoma. However, the majority of our cohort (84.4%) had subtotal resection, while 15.6% had GTR.

The sample size for the GTR group is too small to achieve statistical significance in a multivariate model.

The frequency of grade 3-4 hematological toxicity was higher in our study (46% in combined arm and 43% in extended temozolomide arm) compared to the experimental arm in CeTeG trial (36%), this is because most of our participants completed the chemotherapy courses. Lazaridis *et al*, reported 44% high-grade hematological toxicity in MGMT methylated glioblastoma patients who received CCNU plus TMZ, which was comparable with our results [22]. Lymphopenia and thrombocytopenia frequently occurred in our study in agreement with similar trials [7,20,22].

Grade 3 non-hematological toxicity (nausea, constipation, vomiting and elevated transaminase) was reported in fourteen (15.6%) patients, while In the CeTeG/NOA-09 trial, the authors didn't report higher grade non-hematological events [7]. The prevalence of nausea was higher in extended TMZ arm (6.7%) in agreement with Wang *et al*., who reported higher adverse events in extended group but tolerable [20].

Both the combined and extended arms exhibited notably higher Grade hematological toxicity (46.7 % and 43.3%, respectively), while these regimens show numerical trends toward improved survival, the substantial toxicity profile necessitates careful patient selection and rigorous hematological monitoring. Our protocol prioritized the monitoring of dose-limiting and clinically severe adverse events.

The sample size of 90 patients limits the statistical power of the study, but this study focused strictly on a highly specific molecular subgroup (MGMT-methylated, IDH-wildtype)) within a single institution, the sample size reflects the strict eligibility criteria.

The minimum follow-up of 6 months reflects patients who experienced rapid disease progression and early death, rather than premature drop-out. However early events might explain the long-term survival curves in the more effective treatment arms

The open-label design is a limitation that could theoretically introduce bias. However, to avoid this, radiologic progression was determined strictly using objective RANO criteria.

Our study is the first to compare three lines of treatment (combined temozolomide/CCNU versus standard TMZ versus extended TMZ) for glioblastoma patients with MGMT methylated tumors. The establishment of highly accurate treatment protocols demands comprehensive and detailed studies with larger sample size.

CONCLUSION

For glioblastoma patients with MGMT methylated tumors, particularly younger individuals with good performance status, The combination of temozolomide/ CCNU or extended temozolomide regimen leads to significantly improved survival. While side effects are more frequent, they are generally tolerable.

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CONFLICT OF INTEREST

None.

AUTHORS' CONTRIBUTION

Conceptualization: Fatma Gharib and Asma M Elkady; Methodology: Fatma Gharib, Asma M Elkady, Ahmed El hamouly, and Nehal K.H. Kamel; Formal analysis and investigation: Fatma Gharib, Asma M Elkady, Ahmed El hamouly, and Nehal K.H. Kamel; Writing - original draft preparation: Fatma Gharib, Asma M Elkady, Ahmed El hamouly, and Nehal K.H. Kamel; Writing - review and editing: Fatma Gharib, Asma M Elkady, and Nehal K.H. Kamel; Resources: Fatma Gharib, Asma M Elkady, Ahmed El hamouly, and Nehal K.H. Kamel ; Supervision: Fatma Gharib and Asma M Elkady.

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AGREEMENT TO PUBLICATION

The publication of this study was confirmed by all authors.

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