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TEMOZOLOMIDE IN GLIOBLASTOMA TREATMENT: 15-YEAR CLINICAL EXPERIENCE AND ANALYSIS OF ITS EFFICACY

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***Aim*: To analyze retrospectively the efficacy of temozolomide (TMZ) in various treatment regimens in glioblastoma patients accounting for varying parameters of their treatment. *Materials and Methods:* 302 glioblastoma patients were treated at the State Institution "Romodanov Institute of Neurosurgery of the National Academy of Medical Sciences of Ukraine" from 2003 through 2017. All the patients were surgically treated. In 205 patients, the surgery was followed by adjuvant radiotherapy (RT) with concomitant TMZ (RT + TMZ group). In 97 patients, the surgery was followed by adjuvant RT only (RT group). Kaplan — Meier survival analysis with log-rank test and Cox proportional hazards regression analysis were used for comparing overall survival (OS) and recurrence-free survival (RFS) depending on the age and gender of the patients, the extent of tumor resection, the chemotherapy intensity and the type of RT. *Results:* In RT + TMZ group as a whole, OS median was 20.7 months *vs* 10.8 months in RT group ($p < 0.0001$). The RFS was 14.8 months *vs* 7.9 months, correspondingly ($p < 0.0001$).The survival did not depend on the age, gender or localization of the tumor. On the contrary, the intensity of CTX (the number of TMZ cycles in adjuvant mode), the extent of tumor resection, and the type of RT were among the factors affecting significantly OS and RFS. The improvement in OS and RFS with increasing number of the maintenance TMZ courses was more significant in the patients aged below 60. The use of stereotactic conformal mode for RT provides an advantage in the survival over the conventional RT in RT + TMZ group. *Conclusions*: The combination of concomitant and adjuvant maintenance CTX with TMZ was the most effective CTX regimen affecting positively OS and RFS.**

***Key Words*: glioblastoma, complex treatment, chemotherapy, temozolomide.**

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The incidence of brain cancer has been increasing all over the world within the last 25 years. Such epidemiological trend has been demonstrated convincingly by the Global Burden of Disease Study upon the systemic analysis of the data from 195 countries analyzing cancer cases in 1990–2016 [1]. During this period, the age-standardized incidence of brain tumors increased by 17.3% with the highest indices in Western Europe, East Asia, and central Europe. In 2016, central nervous system cancers caused 227 thousand deaths on a global scale, with age-standardized incidence rate being 4.63 per 100,000 person-years [1]. The global burden of central nervous system cancer has increased. While the mortality rate due to central nervous system cancers did not change globally within the recent 25 years, the improved survival has been demonstrated in the developed countries where the state-of-the-art medical technologies are introduced into the clinical practice [2]. Such data indicate convincingly that the problems related to CNS cancer treatment are in the spotlight from the standpoint of their medical and social significance.

Glioblastoma (GBM), the most common adult brain tumor, accounts for approximately 15% of brain tumors in adults and 46% of the primary CNS malignancies and causes about 100 thousands of deaths in the world population annually [3, 4].

GBM is characterized by the utmost aggressiveness with unfavorable and in most cases lethal outcome. Despite the advances in diagnosis and treatment, the 5-year survival rate remains at the level of less than 10% [5]. Among the risk factors associated with lower survival are the old age of the patient ($\geqslant$ 60 years), the partial resection of the tumor, low preoperative functional status (Karnofsky performance status < 70), the absence of postoperative radiotherapy (RT) and chemotherapy (CTX), less than 4 courses of postoperative CTX with temozolomide (TMZ) [6]. Among prognostically favorable factors are the young age, the radical resection, and the satisfactory general condition of the patient. Some molecular markers such as O^6-methylguanine-DNA-methyltransferase (MGMT) promoter methylation and mutations of isocitrate dehydrogenase (IDH) gene are also considered as prognostically favorable [7, 8].

The survival of GBM patients improved since Stupp et al. [9] introduced TMZ into postoperative RT followed by up to six cycles of adjuvant TMZ. Such regimen allowed for the increase in two-year overall survival (OS) up to 26.5% as compared to 10.4% when only postoperative RT was used in a single mode. Although such treatment regimen is widely used in cases of newly diagnosed GBM, the proper mode of the inclusion of TMZ in the treatment of recurrent GBM is still a subject of controversy [10].

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Abbreviations used: CTX – chemotherapy; DC – dendritic cells; GBM – glioblastoma; HR – hazard ratio; IDH – isocitrate dehydrogenase; MGMT – methylguanine-DNA-methyltransferase; OS – overall survival; RFS – recurrence free survival; RT – radiotherapy; TMZ – temozolomide; TTF – tumor treating fields.

According to the European and North American guidance, the currently accepted standard treatment of the primary GBM comprises the surgical resection followed the adjuvant RT and CTX with TMZ. To increase the CTX sensitivity, the low-intensity, intermediate-frequency alternating electric fields (Tumor Treating Fields — TTF) may also be used [11–13].

Until recently, the standards for the complex treatment of brain tumors with the inclusion of TMZ have not been implemented in Ukraine. One of the reasons is the lack of the data on the effects of different varying factors on the treatment outcome of these patients. Therefore, the aim of our retrospective study was to analyze the efficacy of TMZ in various treatment regimens in GBM patients accounting for varying parameters of the treatment. In particular, the effects of various factors as the predictors of treatment outcome were compared in GBM patients treated postoperatively with RT with or without concomitant and adjuvant TMZ.

MATERIALS AND METHODS

The retrospective study was carried out in the State Institution "Romodanov Institute of Neurosurgery of the National Academy of Medical Sciences of Ukraine" based on the case records and follow-up data of 302 GBM patients treated from 2003 through 2017. Since from the beginning of the study TMZ was not included into the official treatment regimens, the choice of patients seems to be random. The total records were used for the analysis without any selection of the cases among those satisfying the following criteria: age 18–75; confirmed GBM diagnosis; Eastern Cooperative Oncology Group performance status $\leqslant 2$; the anticipated life expectancy $\geqslant 3$ months; the preserved hepatic and renal functions with blood biochemistry within the normal range. The Institutional Review Board approval was obtained to conduct this study.

205 patients were treated surgically followed by adjuvant RT + TMZ (RT + TMZ group). 97 patients were treated surgically followed by adjuvant RT only (RT group). Diagnosis of GBM was verified by pathomorphological study.

The extent of the surgery assessed according to the modified scale was referred to one of the following categories:

- "total" resection (visually complete) — perifocal resection line;
- subtotal resection — resection of 95–99% of tumor mass except for the residual glioma areas associated with the major blood vessels and located in functionally vital regions;
- partial resection — resection of at least 50% of the initial tumor;
- biopsy (stereotactic or open) — resection of less than 50% of the initial tumor sufficient for morphological study.

CTX TMZ was provided according to the standard outlined by The European Organization for the Research and Treatment of Cancer (EORTC) based on the data of the clinical trial 26981 [14].

RT + TMZ group as a whole included the following regimens: 1) RT + concomitant CTX (TMZ in a dose of 75 mg/m^2 on an empty stomach, one hour preceding RT) followed by the maintenance TMZ that started in four weeks after RT (150–200 mg/m^2/d × 5 days, every 28 days);

2) RT + concomitant CTX (the same as in 1) without following maintenance TMZ;

3) RT without concomitant CTX followed by the maintenance TMZ scheduled as in 1.

The accompanying therapy included antiemetics, symptomatic medication, thromboprophylaxis, prevention of thrombocyto- and leucopenia. The use of steroids was considered as a negative factor to the treatment efficacy. When steroids were not required, this was considered as a positive clinical factor.

Postoperative RT was provided to all patients. In most patients, the standard regimen was used (total dose 60 Gy in 30 fractions). In some cases, the total dose was escalated (> 60 Gy in 32–35 fractions). When hypofractionated regimen was used (dose per fraction > 2 Gy), the delivered dose was calculated in terms of the linear-quadratic model, and the biologically effective dose was compared with that of the standard regimen (α/β ratio = 11) [15].

The demographical and clinical data of the patients from both groups are summarized in Table 1.

Table 1. Demographical and clinical data of the patients

Category	RT group	RT + TMZ group	Total
Gender (females/males)	47/50	99/106	146/156
Age			
> 25–44	23	61	84
> 44–60	52	100	152
> 60–75	97	205	302
Localization of tumor			
Right hemisphere	–	89 (43.4%)	89 (43.4%)
Left hemisphere	–	98 (47.8%)	98 (47.8%)
Right + left hemisphere	–	18 (8.8%)	18 (8.8%)
Extent of surgery			
Biopsy	7 (7.2%)	34 (16.6%)	41 (13.6%)
Partial resection	15 (15.5%)	34 (16.6%)	49 (16.2%)
Subtotal resection	34 (35.1%)	67 (32.7%)	101 (33.4%)
Total resection	41 (42.3%)	70 (34.1%)	111 (36.8%)
CTX regimen			
RT + concomitant TMZ followed by maintenance TMZ	–	125 (61%)	125 (61%)
RT + concomitant TMZ without maintenance TMZ	–	39 (19%)	39 (19%)
RT without concomitant CTX followed by maintenance TMZ	–	41 (20%)	41 (20%)
Total dose, Gy			
< 60	0	10 (4.9%)	10 (3.3%)
> 60	0	13 (6.3%)	13 (4.3%)
60	97 (100%)	182 (88.8%)	279 (92.4%)

Statistical analysis. Kaplan — Meier survival analysis was used for estimation of OS and recurrence free survival (RFS). OS was defined from the surgery date to death or loss of follow-up and RFS — from the surgery to progression, death, or loss of follow-up. The survival curves were compared using a log-rank test. The univariate proportional hazard ratio (HR) and its 95% 2-sided confidence interval (95% CI) were computed using the Cox proportional hazards regression model (Cox regression). The right censoring was used in Kaplan — Meier analysis. When OS was analyzed, the lethal event related to the underlying disease was considered as an event type

of 1 while an event type of 0 equaled a right-censored event. The same approach was used for RFS analysis. The time independence of covariates was verified to prove that HRs are unchanged over the time [16].

The data were collected and processed according to the requirements of the national and international standards. For the statistical analysis, the data were prepared and arranged in Microsoft Excel. Statistical analyses were performed using STATISTICA 64 ver. 10.0.1011.0 (StatSoft Inc). For all statistical analyses, two-tailed p values $\leqslant 0.05$ were considered statistically significant.

RESULTS

OS and RFS in RT + TMZ and RT groups. The analysis of the survival in RT + TMZ and RT groups demonstrated significant improvement in RT + TMZ group as a whole. The median OS was 20.7 months for the combined RT + TMZ group and 10.8 months for RT group ($p < 0.001$). The corresponding values for RFS were 14.8 months *vs* 7.9 months ($p < 0.001$). The comparison of survival curves by Kaplan — Meier method is given in Fig. 1.

The analysis by Cox regression demonstrated that in the combined RT + TMZ group the rate of lethal events per unit of time (HR for death) decreased by 41% and recurrence events per unit of time (HR for recurrence) decreased by 30% as compared to RT group.

Effects of covariates on OS and RFS within RT + TMZ and RT groups. The univariate analysis was performed to evaluate the effects of several covariates on the OS and RFS within RT + TMZ and RT groups separately. Among them were the age, the gender, the extent of tumor resection, the localization of the tumor, and the CTX intensity. In each treatment group, neither the age nor the gender affected OS and RFS. The only exception was registered when the combined age group 25–60 years was compared with the group > 60 years (both for OS, $p = 0.0009$ and RFS, $p = 0.0011$).

The effects of the CTX intensity (the number of TMZ cycles) on the treatment outcome were analyzed according to the stratified age groups of the patients within RT + TMZ treatment group using Cox model of the proportional risks. The analysis in the combined group of the patients demonstrated that each added maintenance cycle of TMZ decreased the death risk by 14% (HR = 0.86, 95% CI [0.81; 0.9], $p < 0.001$) and the recurrence risk by 11% (HR = 0.89, 95% CI [0.85; 0.94], $p < 0.001$).

The survival curves for OS and RFS plotted separately for the combined age group 25–60 and the group > 60 depending on the number of the maintenance TMZ CTX cycles (0, 6 or 10) are given in Fig. 2.

The analysis using Cox model of the proportional risks demonstrated that OS relative to the number of CTX cycles is better for the combined 25–60 years age group. While the OS median for the subgroups without maintenance CTX is about the same for the patients aged 25–60 and > 60, for the subgroups comprising 10 cycles of maintenance CTX the median OS in the patients aged 25–60 years exceeds that in the patients aged > 60 by about 10 months. The results calculated for RFS are essentially the same. Therefore, the sensitivity to TMZ in younger than 60 years patients is superior to that in the elderly. According to our calculation, such survival benefit may be estimated as about one month per one CTX cycle.

The factor of tumor localization was not significant for determining the survival in RT + TMZ group ($p = 0.4310$).

The regimens of CTX, namely 1) RT + concomitant TMZ followed by maintenance TMZ; 2) RT + concomitant TMZ without maintenance TMZ and 3) RT without concomitant CTX followed by maintenance TMZ were compared using Kaplan — Meier survival analysis. The differences in the survival between treatment modalities were statistically significant ($p < 0.001$). According to our calculation, OS and RFS in the patients receiving RT + concomitant TMZ followed by maintenance TMZ turned out to be the best as compared to all other treatment modalities (Fig. 3). The difference in efficacy between other CTX regimens was not evaluated in current analysis.

When survival curves for patients in the combined RT + TMZ group or in RT only group were plotted separately for the subgroups of the patients varying by the extent of the surgery, the most favorable OS was found in the patients to whom the "total" resection of tumor was provided ($p < 0.001$ for RT + TMZ group and $p < 0.0001$ for RT group) (Fig. 4).

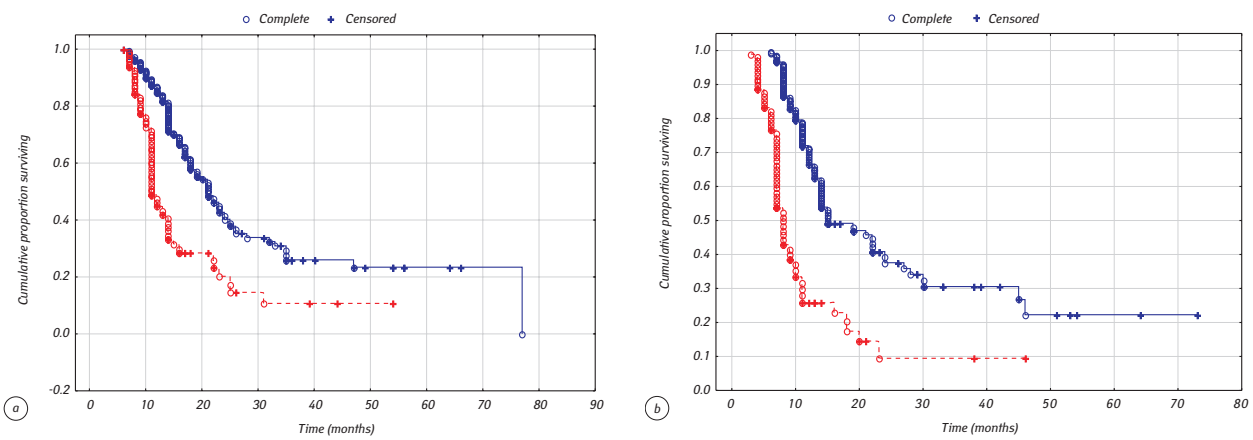

Fig. 1. Kaplan — Meier curves (blue line — RT + TMZ group; red line — RT group) for OS (*a*) (HR = 0.41; 95% CI [0.3; 0.56], $p < 0.001$) and RFS (*b*) (HR = 0.30; 95% CI [0.22; 0.42], $p < 0.001$)

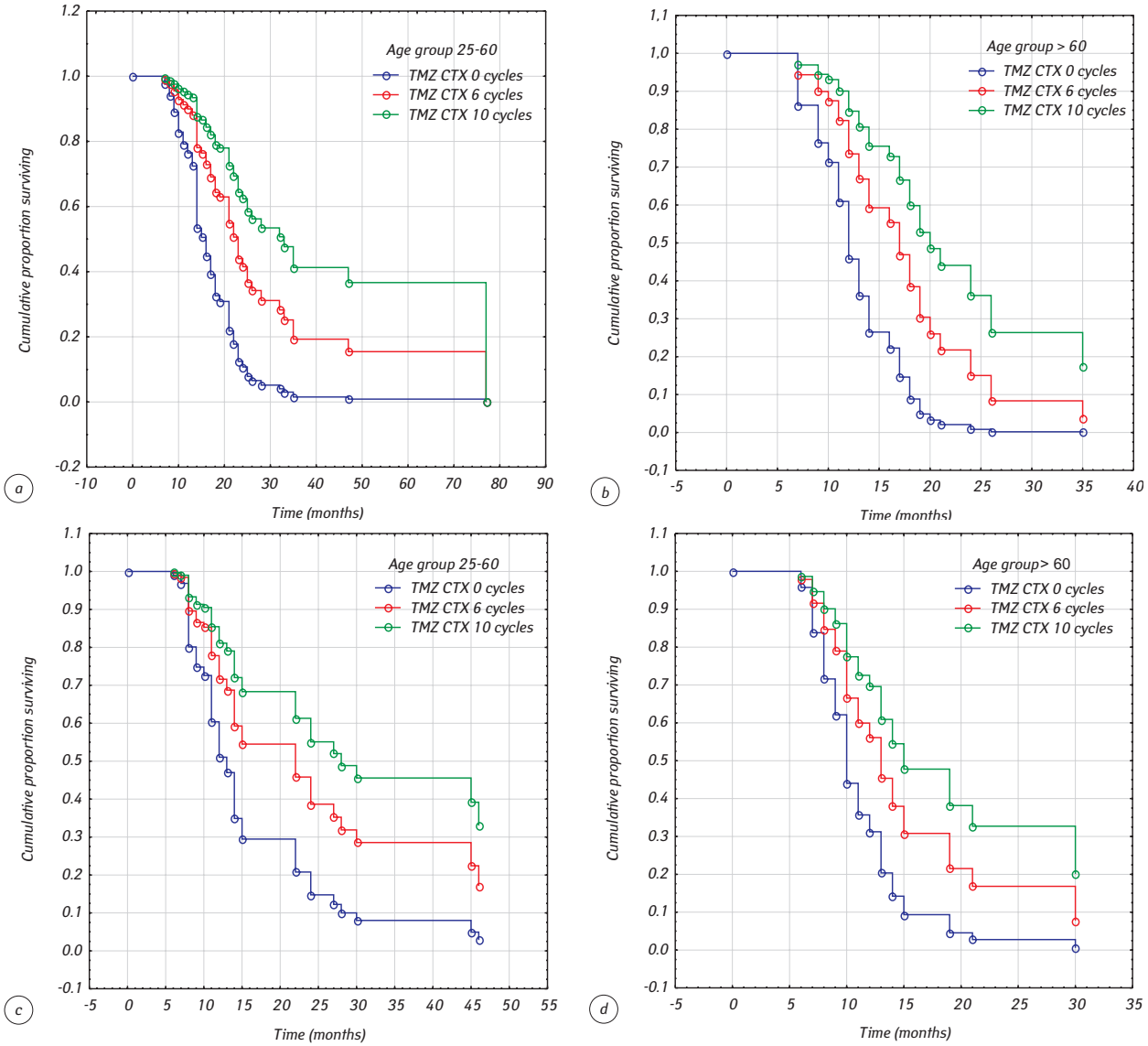

Fig. 2. Cox model calculation of OS (*a, b*) and RFS (*c, d*) of GBM patients by the number of TMZ cycles in the combined age-group 25–60 years (*a, c*) and the elderly group > 60 years (*b, d*)

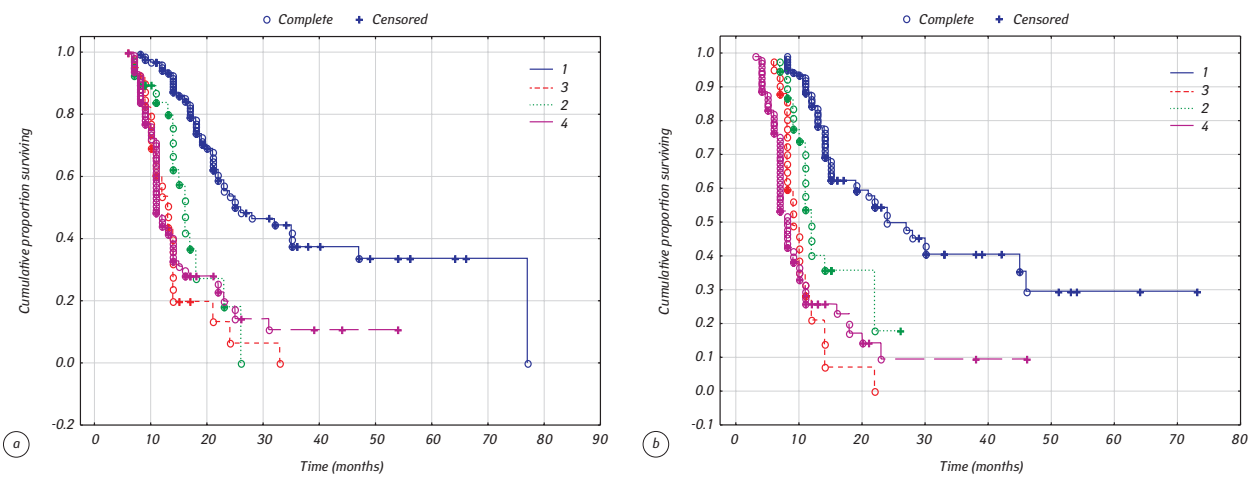

Fig. 3. Kaplan —Meier curves for OS (*a*) and RFS (*b*) of GBM patients treated with different CTX regimens:1 — RT + concomitant TMZ followed by maintenance TMZ (blue line); 2 — RT + concomitant TMZ without maintenance TMZ (green line); 3 — RT without concomitant CTX followed by maintenance TMZ (red line); 4 — RT only (purple line)

Effect of the type of radiation modality on the survival. Throughout the studied period, two modalities of radiation were used with patients being randomly assigned to one or another type. For some patients, the linear accelerator was applied as a source of stereotactic irradiation allowing for the high-conformal dose delivery. The conventional RT was used in other cases. Since both irradiation modalities were applied in both

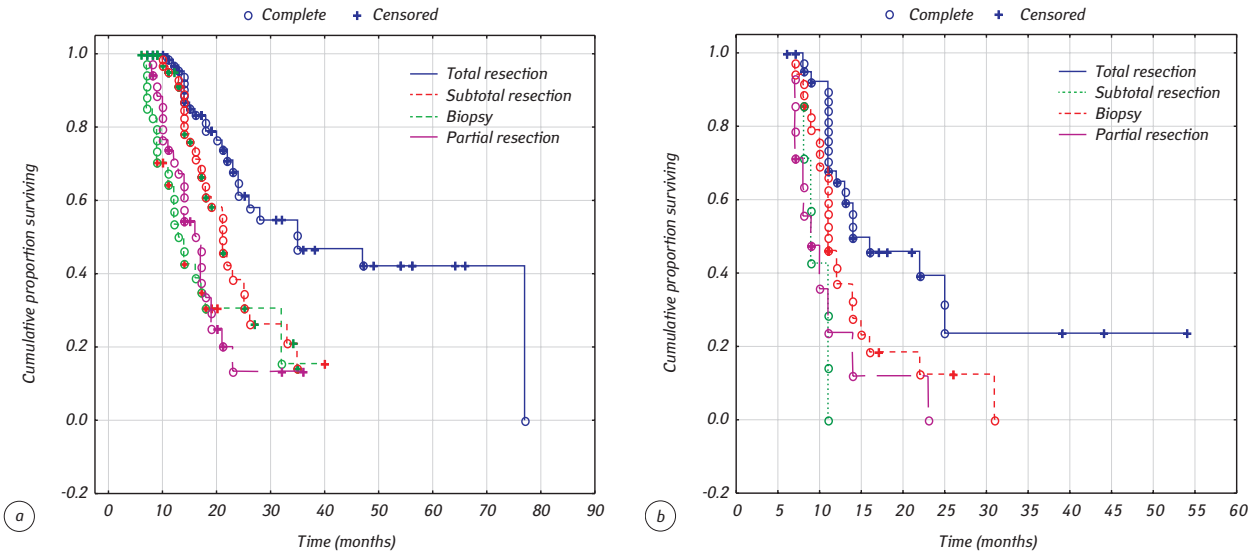

Fig. 4. Association of the extent of the tumor resection with the outcome in combined RT + TMZ group (*a*) and RT group (*b*)

Table 2. Distribution of patients according to the modality of RT

RT modality	RT group		RT + TMZ group		Total	
Stereotactic RT	24	24.7%	149	72.7%	173	57.3%
Conventional RT	73	75.3%	56	27.3%	129	42.7%

RT + TMZ and RT only groups (Table 2), we attempted to analyze the effects of these RT modalities on the survival using Kaplan — Meier survival estimates (Fig. 5).

The significant difference for OS depending on the irradiation modality is evident in RT + TMZ group (p = 0.03) but not in RT group (p = 0.32). Nevertheless, the difference related to the irradiation modality was not significant for RFS (Fig. 6).

Based on the revealed difference in OS of the patients irradiated with different modalities, we attempted to analyze the survival curves plotted separately for RT + TMZ group and RT group in each treatment modality. The results are given in Fig. 7 for OS and in Fig. 8 for RFS.

The analysis of Kaplan — Meier curves allows us to demonstrate the impact of CTX with TMZ in the patients irradiated via both modalities. The difference between RT + TMZ and RT groups was significant both for stereotactic conformal irradiation (p = 0.0117 for OS and p = 0.00465 for RFS) and for conventional RT (p = 0.0096 for OS and p = 0.0001 for RFS).

DISCUSSION

According to the current guidance of the European Association for Neuro-Oncology, National Comprehensive Cancer Network and European Society for Medical Oncology, the concomitant and adjuvant supportive CTX with TMZ combined with RT is a standard for treating the adult patients below 70 years with newly diagnosed GBM and the satisfactory performance and neurological status [11, 12, 17]. Stupp et al. [9] were the first who demonstrated the advantages of the use of RT combined with TMZ for GBM treatment. Based on the data encompassing 573 patients from 85 medical centers, they demonstrated 2.5 months of the median survival benefit and 16.1% increase in two-year survival. In our study, all the patients received TMZ according to the standard outlined by EORTC, namely TMZ was given at a dose of 75 mg/m^2 on an empty stomach, one hour preceding RT followed by the maintenance TMZ started in four weeks after RT (150–200 mg/m^2/d × 5 days, every 28 days) [14, 18].

While in the study by Stupp et al. [9], OS was 14.6 (95% CI [13.2; 16.8]) months for combined therapy *vs* 12.1 (95% CI [11.2; 13.0]) months for RT as a single modality, in our study in RT + TMZ

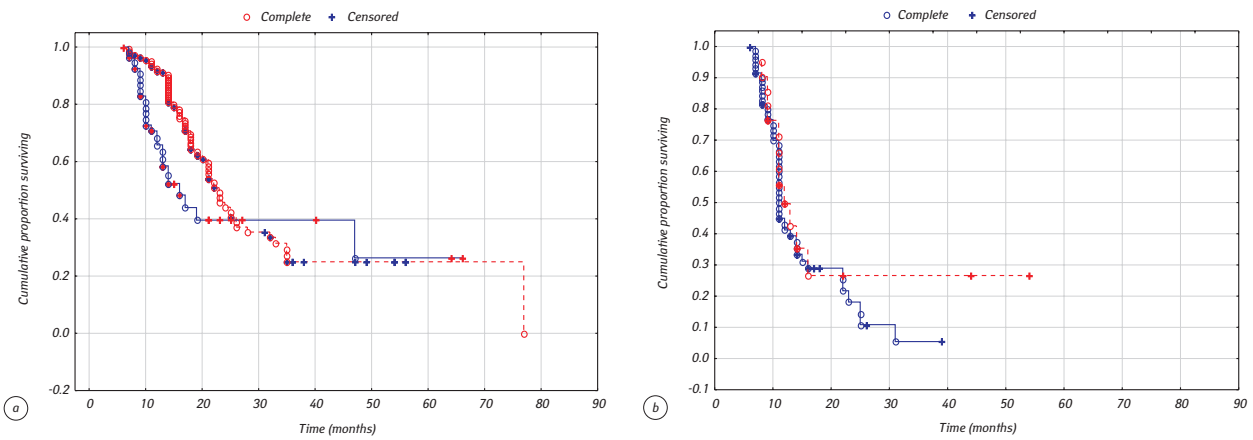

Fig. 5. OS of GBM patients in RT + TMZ group (*a*) and RT only group (*b*). Kaplan — Meier curves were plotted for the stereotactic RT (red line) and the conventional RT (blue line)

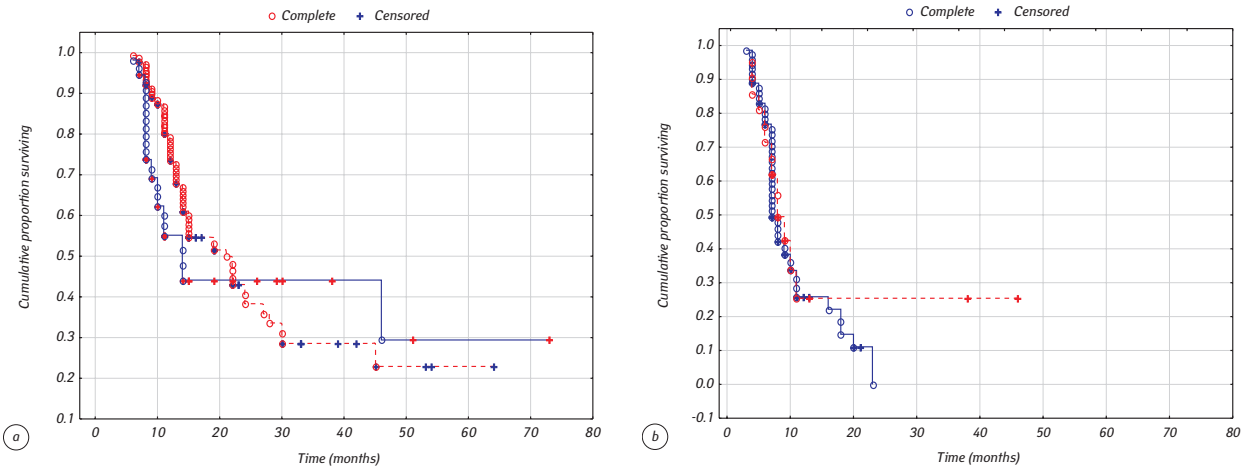

Fig. 6. RFS of GBM patients in RT + TMZ group (*a*) and RT only group (*b*). Kaplan — Meier curves were plotted for the stereotactic RT (red line) and the conventional RT (blue line)

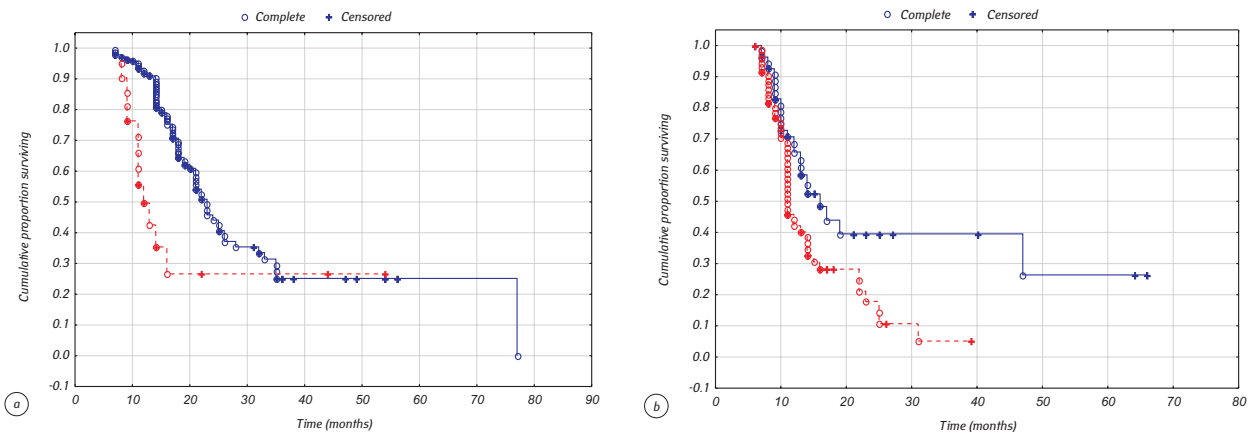

Fig. 7. OS of GBM patients treated with stereotactic RT (*a*) or conventional RT (*b*). Kaplan — Meier curves were plotted for RT + TMZ group (blue line) and RT group (red line)

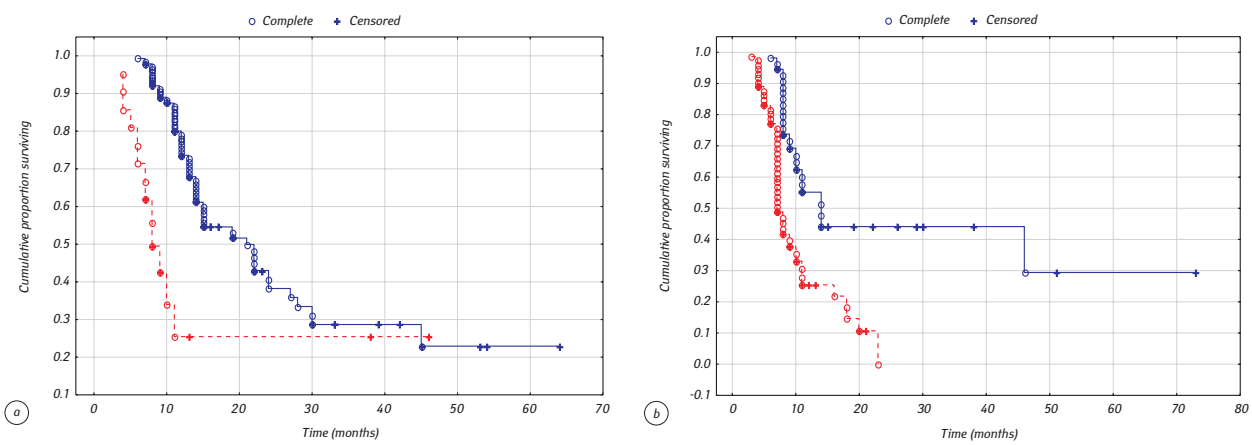

Fig. 8. RFS of GBM patients treated with stereotactic RT (*a*) or conventional RT (*b*). Kaplan — Meier curves were plotted for RT + TMZ group (blue line) and RT group (red line)

group, OS median was 20.7 months *vs* 10.8 months in RT group ($p < 0.0001$). The corresponding figures for RFS were 6.9 (95% CI [5.8; 8.2]) months with RT + TMZ and 5.0 (95% CI [4.2; 5.5]) months with RT alone in Stupp's study and — 14.8 months *vs* 7.9 months ($p < 0.0001$) in the present study.

According to our data, the death probability in RT + TMZ group decreased by 41% (HR=0.41; 95% CI [0.3; 0.56]) and recurrence probability — by 30% (HR = 0.30; 95% CI [0.22; 0.42]) as compared with RT group.

In our study, the survival did not depend on the age, gender or localization of the tumor. The combination of concomitant and adjuvant maintenance CTX was the most effective CTX regimen affecting positively OS and RFS. This trend was also reflected by Stupp et al. [9] who revealed that age and sex did not influence the efficacy of RT + TMZ treatment.

On the contrary, our study showed that intensity of CTX, the extent of tumor resection, and the type of RT were among the factors affecting OS and RFS

significantly. These results differ from Stupp's study where the extent of resection did not appear to influence survival with the exception of biopsy subgroup [9].

The analysis based on the Cox proportional risks model demonstrated the significant decrease in mortality rate per unit of time and recurrence rate with increase in number of the maintenance CTX courses (HR = 0.86, 95% CI [0.81; 0.9]; HR = 0.89, 95% CI [0.85; 0.94] respectively). The improvement in OS and RFS with increasing number of the maintenance TMZ courses was more significant in the patients aged below 60.

The significant association was revealed between the extent of resection and the overall cumulative survival in both treatment groups. The impact of TMZ on OS and RFS was more pronounced in the patients subjected to the total resection of the tumor.

Of special importance is the fact that the modality used for RT affected significantly the survival of the patients especially in RT + TMZ group. The use of stereotactic irradiation provides an advantage in the survival over the conventional RT.

Our high RFS and OS rates of GMB patients can be compared with recent data published by Stupp et al. [19] where TTF in combination with TMZ were used. In this randomized clinical trial RFS median in the TTF + TMZ group was 6.7 months and 4.0 months in the TMZ-alone group (HR = 0.63; CI 95% [0.52; 0.76], $p < 0.001$). The OS median was 20.9 months in the TTF + TMZ group *vs* 16.0 months in the TMZ-alone group (HR = 0.63; CI 95% [0.53; 0.76], $p < 0.001$). TTF involve the focal delivery of low-intensity, intermediate-frequency (200 kHz) alternating electrical fields to the tumor-bearing brain that are postulated to inhibit cell cycle progression through metaphase. Although this treatment is safe and usually well tolerated except local skin reactions, its acceptance by patients, relatives and healthcare professionals is low [18]. Whether the magnitude of OS increase outweighs the individual burden and the social cost is yet to be determined. The long-term relevance of the fields will be determined by whether they are routinely integrated into daily practice and the success (or not) of other concepts of GBM treatment [5].

The fact that higher number of CTX courses (up to 10) positively influenced survival in our study may explain the higher OS and RFS than mentioned by the original Stupp's trial [9]. However, the question of increasing number of CTX courses more than 6 is disputable today [20–22].

We also may speculate that application of stereotactic RT in our study caused less radiotoxicity and could contribute to more significant survival increase than published before [9]. The modern RT technologies allow for minimization of the dose to the critical brain structures (brainstem, chiasma, optic tracts, etc.) and high-conformal dose distribution. The combination of TMZ with hi-tech radiation sources provides an advantage for the patients. This question deserves further study. In general, the results of our study are in line with the data of other studies onfirming the clinical advantages of TMZ use in GBM treatment.

Unfortunately, the outcomes of GBM treatment are generally unsatisfactory worldwide. Therefore, the search for the methods providing effective complex GBM treatment is still on top of the agenda. The abrupt gap between the spectacular conceptual developments and breakthrough at the clinical level is in sharp contrast with the highly limited repertory of the therapeutic modalities available for practical use in the clinics. The studies of the design and development of the effective CTX agents are ongoing. Nevertheless, no less important is the search for the predictive biomarkers of the response to CTX allowing for the improvement of the treatment regimens and overcoming of drug resistance [5, 23].

In AVAREG and EORTC 26101 clinical trials, the association between the *MGMT* status and treatment efficacy has been proved [24, 25]. According to the up-to-date version of WHO classification, the methylation of *MGMT* gene promoter is considered as the predictive feature of TMZ efficacy in GBM treatment [14, 26]. The patients with unmethylated *MGMT* promoter in tumor cells showed only a minor benefit from RT and TMZ treatment, with a median OS of 12.7 *vs*. 11.8 months [27]. According to the European guidelines and the guides of Canadian Cancer Trials Group, TMZ regimens can be adapted according to the methylation status of *MGMT* promoter only in elderly or frail patients. While the tests for such methylation status become more increasing, the results are not taking into account in the management of GBM patients. Nevertheless, testing methylation of *MGMT* promoter may be helpful in detecting the patients whose benefits from TMZ are small allowing for the selection of more appropriate modes of personalized treatment including immunotherapy.

The dose escalation in TMZ treatment of GBM remains the question of high controversy. The antitumor effect of TMZ depends on the schedule with the repeated CTX courses being considered more effective as compared to single one [28]. In other studies, the changes in the standard CTX regimen (21/28 or 7/14 days in adjuvant regimen) or more prolonged courses did not affect the treatment efficacy [29–31]. Jiang et al. [32] demonstrated that super early initiation of TMZ treatment within 7 days after craniotomy might be beneficial for the survival of the patients, especially when the radical resection of the tumor could not be achieved.

According to several observations, about 50% of patients do not respond to TMZ [28]. It is of high importance to understand the mechanisms of the resistance to TMZ. At present, there is no clear understanding of the mechanisms of TMZ resistance that may be mediated by several molecular pathways, in particular the high activity of MGMT and the absence of the functional activity of p53 in response to DNA damage [33–35]. In this respect, the study demonstrating that molecular profile of glioma cells, in particular, methylation status of *MGMT* promoter was not constant throughout the course of the disease, is an important con-

tribution to the analysis of GBM biomarkers [36]. Such changes in the molecular features should be taken into account in planning the personalized treatment.

The growing understanding of GBM biology and the molecular, genomic and epigenomic mechanisms involved in the initiation, growth and progression of this tumor give the hopes for elaborating the therapeutic strategies with more benefits for patients' survival. The heterogeneity of GBM from the point of genetics and cell biology as well as the clinical prognosis has been proved by recent studies that was reflected in the modified version of the 5th edition of the WHO classification of CNS tumors wherein the molecular markers of gliomas are taken into account [3, 13]. *IDH* mutations (R132 of IDH1 and R172 of IDH2) are considered as the positive prognostic markers for the secondary GBM [37]. The existence of GBM with *IDH 1/*2 mutations *de novo* is a point of discussion. The association of *IDH* mutations with better OS and RFS has been reported in many studies regardless of the histological features and the differentiation grade. Therefore, *IDH* mutations seem to be more important prognostic factor than patient's age and the differentiation grade of the tumor [38].

One important aspect of cancer treatment is associated with the immunotherapy. Recently, immunotherapeutic approaches hold the promise for the treatment of metastatic melanoma and lung cancer. Not so long ago, GBM was not regarded as the promising candidate for immunotherapy due to the location of tumors in the immunologically privileged area. Nevertheless, recently such concept was revised substantially following the discovery that macrophages and dendritic cells (DC) are present in microglia and the activated T cells are capable for crossing the blood-brain barrier [39, 40].

The tumor antigen presentation in case of GBM seems to be inadequate due to the general and local immunosuppression, which is characteristic of tumors in general and GBM. Nevertheless, the immune responses might well occur within CNS to efficiently eliminate foreign antigens when properly activated. In particular, the vaccines based on the activated DC as the most potent antigen presenting cells are intensively studied in recent years [41, 42]. Over the past decade, DC-based immunotherapy for CNS tumors has progressed. The promising results were obtained in the clinical trial phase III in the patients with newly diagnosed GBM when OS median of the patients who received postoperatively DC-based vaccine amounted to 23.1 months [43]. Moreover, in the patients with methylated *MGMT* promoter clinical efficacy of the immunotherapy increased substantially.

The recent clinical trials demonstrate convincingly that immunotherapy may be combined successfully with different schedules of RT and CTX. Such combination should not only facilitate the elimination of the tumor cells but also affect the state of the immune tolerance increasing antitumor immune reactions. Such approaches open the new prospects for elaborating the up-to-date concept of radiochemoimmunotherapy with the optimal treatment strategies.

To sum up, our study confirmed the efficacy of TMZ in the complex postoperative treatment of the patients with newly diagnosed GBM. Moreover, the factors affecting the efficacy of TMZ use have been delineated. Among them are the regimen of TMZ application and the extent of the surgical resection of the tumor.

The addition of TMZ to RT early in the course of GBM treatment provides a statistically significant and clinically meaningful survival benefit. Nevertheless, the challenge remains to improve clinical outcomes further. For this reason, the regimen of RT + TMZ should serve as the new platform from which to explore innovative regimens for treating malignant gliomas. Many questions remain unanswered regarding the applications of this regimen to lower grade gliomas and the optimal combination of RT and TMZ. The sensitivity to TMZ in younger than 60 years patients is superior to that in the elderly. For the first time, we have analyzed the impact of TMZ on the efficacy of the treatment when different RT modalities were used.

As further development of our studies, we are planning to analyze the treatment outcomes with TMZ based on the data on the molecular-genetic profiling of the tumors, especially the methylation status of *MGMT* promoter and *IDH* mutations. Another important question is TMZ resistance that should be taken into account in elaborating the strategy of the personalized treatment of GBM patients. The data on the individual radiosensitivity of the patients and their immune status are not less important as the first steps for implementation of the concept of personalized treatment into the clinical practice.

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