Continuous Tamoxifen and Dose-dense Temozolomide in Recurrent Glioblastoma

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Abstract. Background: The current standard-of-care for glioblastoma (GBM) is represented by concomitant radiotherapy (RT) and temozolomide (TMZ), according to Stupp’s protocol. Second-line treatments for GBM have not been yet defined. Tamoxifen is an anti-estrogen molecule with anti-neoplastic effects whose role is under investigation. tamoxifen is generally well tolerated but thromboembolic complications have been reported. In this study, we report our experience on the administration of tamoxifen plus dose-dense TMZ in patients with recurrent GBM. Patients and Methods: All patients underwent surgical resection of GBM and completed concomitant RT and TMZ. Eligibility criteria also included evidence of GBM recurrence and good general conditions [Karnofsky Performance Score (KPS) >70] at recurrence. Patients with rapidly progressive disease, clearly unfavorable prognosis, or history of deep-venous thrombosis were excluded. The second-line treatment consisted of dose-dense TMZ (75-150 mg/m2 one week on/ one week off) plus daily tamoxifen (80 mg/m2). Follow-up was performed with contrast-enhanced brain Magnetic Resonance Imaging (MRI) every three months. Results: Thirty-two patients (18 males, 14 females; median age 57 years) with GBM relapse were included. Median overall survival time (OS) and time to tumor progression after recurrence (TTP-2) were 17.5 and 7 months, respectively. Interestingly, no differences in OS and TTP-2 were noted in GBM between those with methylated and unmethylated MGMT. None of the patients had complications related to TMZ administration. Conclusion: The combinatorial administration of tamoxifen and TMZ appeared to be well-tolerated, and potentially effective in increasing the efficacy of dose-dense TMZ schedule as a second-line therapeutic strategy.

Glioblastoma multiforme (GBM) is an astrocytic tumor displaying a highly malignant behavior (1). Various multimodality treatments have been attempted with the aim of prolonging overall survival (OS) while preserving a good quality of life (QOL). At present, the standard treatment consists of surgical resection followed by concomitant radiation therapy (RT) and temozolomide (TMZ) according to Stupp’s schedule, introduced in 2005 (2). In the work by Stupp et al., patients treated with concomitant TMZ and RT had a median OS of 14.6 months, with median progression-free survival (PFS) of 6.9 months (2). In addition, patients with O(6)-methylguanine DNA methyltransferase (MGMT) methylation had a significantly better OS than those without, probably due to the better response to the chemotherapeutic agent (2). Despite adjuvant treatments, GBM recurrence is expected and is mainly due to its ability to invade white matter tracts and surrounding structures (1). Both disease stabilization and regression are extremely rare events.

At recurrence, second-line treatments have not been standardized and are mainly based on patients’ characteristics and the neuro-oncologists’ experience (3, 4). Different factors can influence the decision for second-line treatment: patient’s compliance, general clinical condition, Karnofsky performance status (KPS), findings at brain magnetic resonance imaging (MRI) and extent of disease progression. Various strategies have been developed and are currently used to treat recurrent GBM. The second-line therapeutic approaches include (but are not limited to) the following: TMZ re-challenge (most frequently with a dose dense scheme), fotemustine, irinotecan or bevacizumab administration (alone or in combination), surgical resection with/without placement of Carmustine polymer wafers and radiosurgery (3-5).

Chronic administration of oral tamoxifen is another approach which has been tested with discordant results (6-14). Tamoxifen is a non-steroidal drug mostly used for its anti-estrogen properties against estrogen receptor-positive...
carcinomas (e.g. in breast cancer). It has been demonstrated that tamoxifen carries out its action on several pathways, not only acting as an inhibitor of estrogen receptor (15, 16). In particular, tamoxifen is an active inhibitor of protein kinase C (PKC), a protein involved in cellular proliferation. Malignant glioma cell lines exhibited an increased activity of PKC when compared with normal astrocytes, and this was shown to be related to tumor proliferative ability (17, 18). The inhibitory action of tamoxifen on PKC was associated with an increase in cellular apoptosis and with a reduction of DNA production in a dose-dependent manner in in vitro studies (15). It has also been shown that high-dose tamoxifen can reverse the chemoresistance induced by ATP-binding cassette, subfamily B (MDR/TAP)-1 (ABCB1) gene in vitro (19). Finally, it has been shown that the de-differentiation processes of neoplastic cells in astrocytic brain tumors involve estrogen receptors (20).

Some researchers tested the use of tamoxifen as an anti-neoplastic agent against malignant glioma (7-10, 14) with encouraging results in some cases (7, 12). However, no standardized protocol for the use of tamoxifen in the treatment of high-grade gliomas is available.

Considering the lack of clearly positive or negative literature reports and the overall minimal side-effects, we tested the use of continuous oral administration of tamoxifen in association with TMZ as a second-line treatment for patients with recurrent GBM. The aim of the study was the assessment of OS and time to tumor progression after recurrence (TPP-2) in patients receiving dose-dense TMZ and oral high dose tamoxifen at GBM recurrence. We report our experience on a group of 32 patients with recurrent GBM treated at our hospital between 2006 and 2010 and followed-up until September 2012.

Patients and Methods

Patients. Thirty-two patients with recurrent GBM were included in the study and selected according to the inclusion and exclusion criteria below from a consecutive series of 100 cases of GBM treated at our hospital between 2006 and 2010. MGMT status at diagnosis was assessed using methylation-specific PCR (MSP) according Hegi and colleagues (21). The last follow-up was performed in September 2012.

Table I. Elegibility and exclusion criteria for inclusion in the study of treatment with dose-dense temozolomide-plus-tamoxifen at first relapse of glioblastoma.

<table>
<thead>
<tr>
<th>Eligibility criteria</th>
<th>Exclusion criteria</th>
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<tbody>
<tr>
<td>Patients underwent RT plus TMZ according Stupp’s schedule</td>
<td>Evidence of pseudoprogression or radionecrosis</td>
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<tr>
<td>Complete or partial response to Stupp’s schedule</td>
<td>Patient not compliant with treatment/ absence of consent</td>
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<tr>
<td>Evidence of GBM recurrence at follow-up brain MRI</td>
<td>Multifocal recurrence/ rapidly disease progression</td>
</tr>
<tr>
<td>KPS ≥70 and age &gt;18 years</td>
<td>KPS &lt;70; age &lt;18 years</td>
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<tr>
<td>Life expectancy &gt;3 months</td>
<td>Life expectancy &lt;3 months</td>
</tr>
<tr>
<td>Consent to the treatment</td>
<td>Thrombophilic conditions or past thromboembolic events</td>
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Inclusion and exclusion criteria. Inclusion and exclusion criteria are summarized in Table I.

Eligibility criteria were evidence of GBM recurrence at brain MRI after RT and TMZ according to Stupp’s schedule; histological confirmation of recurrent disease was obtained in 6 patients who underwent a second surgical resection. Whenever indicated, differential diagnosis with radionecrosis or pseudoprogression was performed using MR spectroscopy or methionine-positron emission tomography (PET). Good general conditions with a KPS>70 at recurrence, age greater than 18 and life expectancy >3 months also had to be met for inclusion in the study.

Exclusion criteria included evidence of rapidly progressive disease, history of deep venous thrombosis or other thrombophilic conditions (e.g. central venous catheter, genetic predisposition etc.), inadequate patient compliance and multifocal GBM/recurrence. Finally, patients who did not respond to Stupp’s schedule were excluded.

Written informed consent to the therapy with tamoxifen was acquired before starting treatment with tamoxifen.

Treatment protocol. All patients had first-line treatment consisting of surgical resection of the brain lesion followed by RT and TMZ, according to Stupp’s schedule (2). For all patients, at tumor recurrence, the second-line treatment was dose-dense TMZ plus tamoxifen (see below). In six cases (three with MGMT methylation and three without), patients underwent also a second surgical removal, with positioning of at least four carmustine wafers per patient.

The treatment at tumor relapse consisted of TMZ dose-dense at 75-150 mg/m² one week on/one week off plus daily tamoxifen at 80 mg/m². This schedule of TMZ was selected according to the patient’s medical conditions and hematological examinations after the first-line treatment. Two patients developed mild thrombocytopenia after the first-line treatment with TMZ. In these cases, it was decided to reduce the dosage of TMZ. Second-line therapy was administered until further tumor relapse.

In those cases in which, at the time of tumor recurrence, a contrast-enhancing lesion with mass-effect was detected, patients were proposed a second surgery before starting second-line treatment with dose-dense TMZ plus tamoxifen and, when possible, carmustine wafers (Gliadel®; Eisai Inc. Woodcliff Lake, NJ, USA) were placed in the surgical cavity.

Follow-up and screening of toxicities. Patients underwent regular follow-up for GBMs which consisted of neuro-oncological assessment and brain MRI with gadolinium every two to three months; in cases
of any clinical modification or onset of new symptoms, the MRI and the clinical assessment were performed earlier.

Regular blood tests were performed for screening toxicities related to TMZ. In addition to regular clinical examination, specific attention was paid to the possible development of thromboembolic complications (deep venous thrombosis, pulmonary embolism, cerebral ischemia) or metrorragic events due to tamoxifen administration.

Tumor progression was defined according to the radiological criteria described by Macdonald et al. (22).

Results

Patients population. The median age of the 32 patients included in the study was 57 (range=39-75 years). Eighteen patients were male and 14 were female. The median KPS at presentation was 80 (range=60-100).

All patients underwent surgical resection prior to concomitant Stupp’s schedule: gross total resection was achieved on 16 patients, subtotal resection in five, while 11 patients underwent a partial resection. None of the patients underwent a purely bioptic procedure. Six patients underwent a second surgical resection at recurrence and prior to being included in the study.

Histological analysis revealed GBM (WHO grade IV) with an average Ki-67 index of 10%. The MGMT gene was methylated in 20 patients and unmethylated in 12.

Treatment response rate, tumor progression time and survival time. The response rate to dose-dense TMZ plus high dose continuous tamoxifen was the following: no response in 18 patients with methylated MGMT, the median OS time was 20.5 months, in those with unmethylated MGMT the median OS time was 15 months.

The patients included in this study had a median OS time of 17.5 months. In patients with methylated MGMT, the median OS time was 20.5 months, in those with unmethylated MGMT the median OS time was 15 months. Interestingly, this difference in OS was not statistically significant by Gehan-Breslow-Wilcoxon test (p-value=0.10) (Figure 1).

The median time to tumor progression (TTP-1) was 9.5 months. Patients with MGMT methylation had a median TTP-1 of 11 months, while those without had a median TTP of 7.5 months. This difference, in agreement with previously published studies, was statistically significant by the Gehan-Breslow-Wilcoxon test (p-value=0.04) (Figure 2).

At further progression, after treatment with dose-dense TMZ plus tamoxifen, the median TTP-2 was 7 months. After the first relapse and dose-dense TMZ plus tamoxifen treatment, the median TTP-2 was 6 months for patients with MGMT methylation and 7 months for those without. This difference in TTP-2 was not statistically significant by Gehan-Breslow-Wilcoxon test (p-value=0.75) (Figure 3).

The patients who underwent a second surgical resection had a median TTP of 10 months. No significant difference in TTP-2 and OS was noted in this small subgroup of patients (p-value=0.14; 0.4, respectively).

Five (15.6%) patients developed multiple contrast-enhancing lesions (between two and four). One of these patients developed a lumbar metastasis after 28 months of tamoxifen treatment while there had been a partial response on brain MRI.

Median OS, TTP-1 and TTP-2 are reported in Figures 1, 2 and 3, respectively, as Kaplan-Meier functions.

No differences between males and females patients was found (p>0.05) and no differences in terms of OS, TTP-1 and TTP-2 were found between patients that underwent a second-look surgery and patients who underwent to only one surgery.

Complications. No toxicity was observed in the 32 patients included in the study and all the patients were able to continue the chemotherapeutic regimen until further progression.

No thromboembolic events or other complications related to tamoxifen administration were noted.

Discussion

The introduction of oral administration of TMZ with concomitant RT has radically changed the treatment strategy for GBM, increasing TTP and OS (2). Since then, the first-line treatment for GBM has been standardized, obtaining a median survival time of at least 14 months in different series (23). On the contrary, second-line therapies have not been homogenously applied due to lack of evidence on this topic and to the poor prognosis of patients with GBM at tumor relapse. Thus, it is currently difficult to choose the optimal approach for individual patients.

In the literature, the use of TMZ as second-line treatment is a relatively well-established strategy with different authors reporting on its efficacy with a dose-dense schedule (3-5). As for what the use of tamoxifen is concerned, published data appear more confusing. Chronic low-dose (20 mg b.i.d.) oral administration of tamoxifen failed to demonstrate a significant increase in OS at GBM relapse (6). Thus, subsequent studies were based on higher doses of tamoxifen (7-10, 14, 24). Couldwell et al. suggested the use of chronic oral high-dose of tamoxifen for patients with anaplastic astrocytomas or GBM. In their study, maximum doses of 160 mg/day of tamoxifen for female patients and 200 mg/day for males where achieved. Median OS in patients with GBM was 17.4 months. In particular, clinical and radiological stabilization in a subgroup of patients was noticed, some of whom had failed standard chemotherapy with nitrosoureas. These findings defined the potential usefulness of chronic high-dose administration of tamoxifen (7). Such promising results were confirmed by...
Puchner et al. who studied the role of tamoxifen in association with carboplatin and RT after surgery for newly-diagnosed GBMs. Fifty patients were enrolled and they were administered 200 mg of tamoxifen daily, while carboplatin was administered at 300 mg/m² per day for three cycles. RT was started after the last carboplatin cycle. The 6-month survival rate was 90%; it was 58% at 12 months and 34% at 18 months, while at 24 months, the rate was 18% (9). In this phase II trial, the authors observed that a subgroup of patients had a significantly longer TTP and OS. In particular, females had a better outcome than males (29% living longer than two years) (9). A higher incidence of multifocal tumor recurrence in patients treated with tamoxifen was noted (33% compared with 4-14% reported in the literature) (25).

In our series, the combinatorial administration of continuous high-dose tamoxifen (80 mg/m²) plus dose-dense TMZ at GBM recurrence led to an OS of 17.5 months and to a TTP-2 of 7 months. The latter result, being clearly affected by the second-line therapy administered, appeared promising to us. Most importantly, we noted that MGMT methylation at first relapse did not affect OS or TTP-2 (Figures 1 and 3). This finding was intriguing considering that TTP-1 was significantly different between patients with and those without MGMT methylation, thus suggesting that the lack of difference in TTP-2 and OS might be due to the second-line treatment chosen in this study. Nevertheless, it is also possible that at recurrence, methylation was lost by some of the GBMs. Finally, we did not find a significant difference between males and females in TTP and OS, nor in the response to tamoxifen administration ($p > 0.05$ in all analyses).

As for tamoxifen toxicity, Spence et al. reported unsatisfactory results on the use of tamoxifen plus TMZ...
against recurrent malignant astrocytoma after surgical resection; in fact, their study was interrupted due to low response rates and a high frequency of toxicity (10). Median survival was 26 weeks and complicated by several toxicity-related events, such as pancytopenia, herpes zoster and deep-venous thrombosis. Conversely, according to other studies, chronic oral high-dose tamoxifen is a reliable treatment option in patients with recurrent GBM (7-9, 11-14). The most commonly reported severe adverse event consisted of deep-venous thrombosis associated with pulmonary embolism. None of the patients enrolled in our study experienced thromboembolic complication. This might be due to the eligibility criteria of our study (Table I), which included patients with good KPS (median KPS=80) and excluded patients with history of previous thromboembolism (or at high risk for it).

As for the reported high rates of multifocal recurrence after tamoxifen administration (23), we observed multifocal recurrences in only five patients (15.6%), one of them had spinal seeding of GBM after 28 months of treatment. Thus, our data did not confirm Puchner et al.’s results and are in agreement with the current literature reports where multifocal relapses range from 8.75 to 20% (26-28).

Critically analyzing the satisfactory results of the proposed scheme of second-line therapy (dose-dense TMZ plus tamoxifen), we should consider the following: firstly, the eligibility criteria for this study included factors recognized as positive prognosticators. In fact, our patients had a good KPS, satisfactory surgical resection (none had a simple biopsy and 67% had a total or subtotal resection) and multifocal relapses were excluded. Secondly, the enrolled patients had complete or partial responses to Stupp’s schedule (see Table I) (3, 4, 23). Another consideration regarding our therapeutic schedule is that the majority of the studies reported in literature tested tamoxifen on patients treated with other antineoplastic agents rather than TMZ or tamoxifen alone (see Table II) (6-9, 11-14). The only study in which tamoxifen was administered in association with TMZ in patients with recurrent malignant astrocytoma was interrupted because of low response rates and serious toxicity-related complications (10). These adverse events could be due to the prolonged administration of TMZ (six weeks with no interruptions). In our protocol, TMZ was administered in a week on-week off schedule, it was well-tolerated and in none of our cases did tamoxifen treatment need to be suspended or delayed. Moreover, tamoxifen did not increase toxicity, rather showing an improved antineoplastic effect.

Finally, Patel et al. recently proposed the use of daily tamoxifen during Stupp’s schedule in newly-diagnosed GBM: a median OS of 17 months was reached, with 35% of patients alive after two years (29). This last work, reporting an OS similar to ours, is further confirmed by our favorable experience with the use of tamoxifen in association with TMZ. Considering their results, and the data deriving from the present study and other works on tamoxifen and TMZ, we believe that it is still necessary to establish a standard dose of tamoxifen and that neuro-oncologists should design studies to assess whether an association of tamoxifen plus TMZ may be administered during first-line or second-line treatment of GBM.

**Conclusion**

Our experience in second-line treatment of recurrent GBM with dose-dense TMZ plus tamoxifen confirmed that oral high-dose administration of tamoxifen was well-tolerated and easily used. Moreover, this study confirms the hypothesis that continuous administration of tamoxifen combined with dose-dense TMZ significantly improves progression-free survival and overall survival compared to TMZ alone.
that oral high-dose tamoxifen associated with dose-dense TMZ can increase OS and TTP in patients with recurrent GBM. Patients with unmethylated MGMT might benefit more from this combined regimen.

We believe that further prospective, and eventually randomized, studies are needed in order to clarify the efficacy and validate this regimen as standard therapy at GBM relapse in a first-line setting to improve therapy outcome.

Conflicts of Interest

None of the Authors have any conflicts of interest.

References

10 Spence AM, Pateron RA, Schramhorst JD, Silbergeil DL and Rostomily RC: Phase II study of concurrent continuous


