Acta Scientific Cancer Biology

Volume 1 Issue 2 July 2017

Research Article

Effect of DNA Methyltransferase Resistance to Temozolomide in Gliomas or Brain Metastases of Melanoma

Emad Y Moawad*

Researcher Graduated from Ain Shams University, Department of Engineering, Cairo, Egypt

***Corresponding Author:** Emad Y Moawad, Researcher Graduated from Ain Shams University, Department of Engineering, Alhegaz Street, Alnozha, Cairo, Egypt.

Received: June 06, 2017; **Published:** September 07, 2017

Abstract

The aim of this study is to investigate the resistance of DNA methyltransferase (MGMT) in gliomas or brain metastases of melanoma to Temozolomide (TMZ) therapy. The *in-vivo* effects of standard, metronomic and dose-dense TMZ regimens in animal models were monitored to identify efficacy of those regimens with respect to suppression of MGMT-resistance in tumor cells. MGMT-resistance is dependent mainly on the TMZ dose/day of the applied regimen, observed when applying standard regimens of 2.45 mg/kg bw/day for 5 days every 28 days and maximized by increasing dose/day to 6.74 mg/kg bw/day for 5 days every 28 days. Afterwards, MGMT-resistance decreased gradually until being suppressed by increasing the received dose/day in standard regimen to 8.88 mg/kg bw/day for 5 days every 28 days. Standard regimen of dose less than 4.37 mg/kg bw/day for 5 days every 28 days is more efficient than the metronomic one of dose/day less than 0.78 mg/kg bw/day for 28 days in early stages of primary tumors.

While the metronomic regimen of dose/day lies between 0.78 and 1.6 mg/kg bw/day for 28 days is more efficient than the standard one of administered dose lies between 4.37 and 8.96 mg/kg bw/day for 5 days every 28 days in the moderate stages of recurrent tumors of higher MGMT-resistance. Dose-dense regimens with standard schedule of dose/day higher than 8.96 mg/kg/bw/day for 5 days every 28 days or metronomic schedule of dose/day higher than 1.6 mg/kg bw/day for 28 days suppress MGMT-mediated resistance in advanced stages of high-grade tumors by depleting MGMT in tumor cells.

Keywords: Temozolomide; O^6-Methylguanine-DNA Methyltransferase; Gliomas; Brain Metastases of Melanoma

Introduction

Temozolomide (TMZ) is an imidazotetrazine derivative of the oral alkylating agent dacarbazine with established antitumor activity in patients with primary and recurrent brain tumors and brain metastases of melanoma [1]. It undergoes rapid chemical conversion in the systemic circulation at physiological pH to the active compound, MTIC (monomethyl triazene imidazole carboxamide) [2]. In a recent randomized trial, concomitant and adjuvant TMZ chemotherapy with radiation significantly improves, from 12.1 months to 14.6 months, progression free survival and overall survival in glioblastoma Multiforme (GBM) patients [3]. The therapeutic benefit of TMZ depends on its ability to alkylate/methylate DNA, which most often occurs at the N7 or O6 positions of guanine residues. This methylation damages the DNA and triggers the death of tumor cells [4]. Unfortunately, TMZ is not always effective in patients with gliomas; in some cases, tumor cells are able to repair this type of DNA damage, and therefore diminish the therapeutic efficacy of TMZ due to an enzyme called O6-methylguanine-DNA methyltransferase (MGMT) or O6-alkylguanine-DNA alkyl transferase (AGT or AGAT) [5-7]. While in other cases of responding tumors to TMZ therapy, epigenetic silencing of the MGMT/AGT gene prevents the synthesis of this enzyme, and as a consequence such tumors are more sensitive to killing by TMZ [8]. Thus, the increase of MGMT in brain tumors during TMZ therapy predicts poor response and these patients receive little benefit from chemotherapy with TMZ [9]. Such unsatisfactory outcome of chemotherapy with TMZ is mainly defined by the intrinsic or acquired MGMT-resistance repairing DNA of tumor cells. Several trails have been conducted to overcome TMZ resistance acquired by recurrent GBM or High-Grade Glioma (HGG) including reversed approaches and the use of therapeutic agents to suppress the activity of MGMT in tumor cells. However, those trails had adverse effects as impaired hepatic and renal function or myelosuppression [10,11]. Since TMZ exhibits schedule-dependent antineoplastic activity by interfering with DNA replication, then a better understanding of the relationship between the MGMT-resistance and the efficacy of the applied TNZ regimen could help to design a new therapeutic regimen to overcome MGMT-resistance to TMZ. The originally approved TMZ dosing regimen is 150 to 200 mg/m2 per day on days 1 to 5 of each 28-day cycle (5 of 28 days) as a clinically active and generally well-tolerated regimen [12-17]. Tolcher., et al. showed that MGMT

enzyme activity consumed during treatment with TMZ for either 7 consecutive days every 14 days (7 of 14-day regimen) or 21 consecutive days every 28 days (21 of 28-day regimen). These extended dosing regimens allow for administration of a higher cumulative dose per cycle and have been shown to deplete O6-methylguanine-DNA methyltransferase, which may enhance cytotoxic activity [18]. Thus, these schedules of dose-dense regimens that deplete MGMT were suggested to improve antitumor activity against MGMT-resistance in tumor cells. On the other hand, the antitumor activity of low dose metronomic (LDM) regimens in the TMZ therapy has gradually been elucidated by preclinical and clinical research suggesting that the resistance of recurrent tumors to TMZ can be overcome by synchronizing metronomic TMZ regimen with MGMT resistance. However, the extent of MGMT depletion in tumor cells in each type of the proposed regimens has not been directly demonstrated yet. Current thesis identifies the dosing limits of TMZ to overcome MGMT-resistance, investigates the efficacy of those regimens and recommendations to all stages of primary or recurrent tumors for optimizing therapy.

Methods and Materials

Standard and LDM TMZ Regimens in Rats Bearing C6/Lacz Rat Glioma Cells and Mice Bearing U-87MG Human Glioblastoma Cells

(1) As conducted and described by Kim., *et al.* [19]; Rat glioma cell line, C6/LacZ, and U-87MG human glioblastoma cells (1 x 10^5 cells/10 µl of Hanks balanced salt solution) were injected at a depth of 5 mm for male Sprague-Dawley (SD) rats (200 - 250 g) and male Balb/c-nu mice (6 weeks) respectively, through a 10-µl Hamilton syringe connected to the manipulating arm of the produced 7-stereotatic device. All injections consisted of a total volume of 10 µl delivered over 12.5 min by a micro infusion pump. Before treatment of TMZ, the rats were randomized into four groups (n = 10 per group). One experimental group of rats was administered orally with 7 mg/kg TMZ for 5 days (between day 7 and 11 after intracranial implantation) to represent a tolerated synchronized standard regimen for TMZ therapy. Two groups of rats were respectively administered 1 or 2 mg/kg TMZ via per os (p.o.) every day for 16 days to represent a tolerated LDM regimen for TMZ therapy. The control group of rats was treated by injection via p.o. with 10% DMSO. Human U-87MG at nude mice brain is so sensitive to TMZ then the starting dose of TMZ was minimized to show the antitumor activity. The mice were randomized into five groups (n = 15 per group). Two conventionally-treated groups of mice were administered orally with 2.5 or 1.25 mg/kg TMZ for 5 days, respectively (between day 14 and 18 after intracranial implantation) to represent a tolerated standard regimen for TMZ therapy. Two groups of mice were treated orally with each dose of TMZ (0.5 and 0.25 mg/kg) daily for 25 days to represent a tolerated LDM regimen for TMZ therapy. The control group of mice was treated by p.o. with 10% DMSO. Seventeen and twenty-five days after the inoculation of tumor cells, rats and mice were anesthetized and sacrificed respectively. The tumor volume was calculated by measuring the section with the largest tumor portion and applying the formula: Length x Width2 x 0.5.

(2) As conducted and described by Son., *et al*. [20]; briefly, male SD rats (200 - 250g) were anesthetized and shaved. They were secured in a rodent stereotactic frame, and a hollow guide screw was implanted into a small drill hole made 3 mm left lateral and 1 mm anterior to the bregma [21]. C6/LacZ rat glioma cells (1 x 10^5 cells/10 µl) were injected through this guide screw into the white matter at a depth of 5 mm.

TMZ (7.5 mg/kg) was administered intraperitoneally for 5 days, between the 7^{th} and 11^{th} day after tumor cell inoculation to represent a synchronized tolerated standard regimen for TMZ therapy.

Dose-Dense Regimen in Mice Bearing Human Melanoma UACC903 Tumor Model

As conducted and described by Zhang L., *et al*. [22], Melanoma mouse model: Nude mice (swissnu/nu) were inoculated s.c. with UACC903 human melanoma cells (1×106 cells/100 µl/mouse). When the tumors reached 100 mm3 in volume, the mice were treated i.p. with TMZ (15 mg/kg) on days 1, 3, 5, 7 and 9 to represent a dose-dense regimen for TMZ therapy. The control group of mice was treated by p.o. with 10% DMSO. Tumor sizes and body weight of the animals were measured every other day. The differences between treated and control groups were analysed using a two-sample t-test. The survival curves of the treated tumor - bearing mice were estimated using Kaplan-Meier method and compared by log-rank statistical analysis.

Monitoring the Mechanical Behavior of the Tumor Response to Therapy

Comparing the mechanical behavior of tumor response of the treated groups by that of the control groups is assessed by determining the growth/or shrinkage constants of those tumors of different volumes along the corresponding periods [23,24]. The tumor growth/or shrinkage constant at a certain time expresses the rate of the difference between Mitosis and Apoptosis with respect to the total number of the tumor cells (M - A) that characterize the tumor response at that time [23-27]. If rate of mitosis is greater than that of apoptosis, tumor grows by growth constant of $\frac{\ln 2}{t_D}$, and vice versa if rate of mitosis was less than that of apoptosis, tumor shrinks by shrinkage constant of $\frac{\ln 2}{t_{1/2}}$, where is the tumor half-life time [23, 27].

i.e. $(M - A) = \frac{\ln 2}{t_D}$ S^{-1} in case of tumor growth,

&

$(A - M) = \frac{\ln 2}{t_{1/2}}$ S^{-1} in case of tumor shrinkage,

where t_D and $t_{1/2}$ in seconds Equation (1).

The clinical staging model presented by Moawad showed that the tumor histologic grade (H_G) that expresses tumor response can be identified by using Emad formula [28-46] as follows:

In case of tumor growth

$$H_G = \ln\left(\ln\frac{\ln 2}{t_D}\right)^2 \times C_0 \times h \times 23234.59 \text{ MeV}$$ Equation (2),

where $C_0 \times h$ is number of the hypoxic cells in the tumor or number of the inoculated cells in the transplanted tumor in xenografted models [25-40].

In case of tumor growth

The chemotherapeutic drugs affect the tumor cells such that the more the drug dose the less of mitotic cells or the more of apoptotic cells. Since the portion of tumor cells underwent apoptosis due to chemotherapeutic growth inhibitors had been prevented first from mitosis. Thus, to apply equation 2 in the shrinkage case, the apoptotic tumor portion of half-life time ($t_{1/2}$) would be replaced by the growth portion of doubling time (t_D) which had been prevented first from mitosis whose rate of growth is inversely proportional to the rate of the shrinkage of the apoptotic portion as follows:

$$\left(\frac{V_{Initial} - V_{Final}}{V_{Initial}}\right)_{Shrinkage} = \left(\frac{V_{Initial}}{V_{Final} - V_{Initial}}\right)_{Growth}$$

Equation (3) [25-27, 40]

Accordingly, from Equations (1) and (2) the alteration in the treated tumor from that of the control tumor induced by the drug dose would be equivalent to the energy yield by the drug dose according to the following model:

$$E_{Dose} = \left[\ln\left(\ln(M - A)_{Treated}\right)^2 - \ln\left(\ln(M - A)_{Control}\right)^2\right] \times C_0 \times h \times$$

23234.59 MeV Equation (4) [25-27,35-40].

Results and Analysis

To Evaluate the *In Vivo* Activity of TMZ in the LDM Regimens

In rats: LDM regimens of TMZ inhibit the tumor growth in C6/LacZ rat glioma orthotopic model in a dose-dependent fashion after daily dosing for 16 days; the average tumor size of control group grew from 10 mm^3 at the implantation to 100 mm^3 (n = 10 , P < 0.001) in 17 days [19] with doubling time (t_D) of 5.12 day. While, the average tumor size of the two treated groups by daily dose of 1 and 2 mg/kg for 16 days grew from 10 mm^3 to 44.09 and 29.73 mm^3 (n = 10 , P < 0.001) in 17 days [19] with of 7.94 and 10.82 days respectively. TMZ doses of 1 and 2 mg/kg/d for 16 days in human (70 kg, 2.5L plasma) are equivalent to ([1 or 2] 16 70 mg/2.5L) 448 and 896 μg/mL respectively. Thus from Equation 1 and 4, the energy yield by 448 and 896 μg/mL of TMZ in LDM regimens ($E_{TMZ\ in\ LDM}$) in tumor xenograft of intravenously transplanted 1×10^5 C6/LacZ rat glioma cells were equivalent to: $= E_{TMZ(448\ \mu g/mL)\ in\ LDM}$

$$\left[\ln\left(\ln\left[\frac{\ln 2}{7.94\times24\times60\times60}\right]\right)^2 - \ln\left(\ln\left[\frac{\ln 2}{5.12\times24\times60\times60}\right]\right)^2\right] \times 1 \times 10^5 \times$$

$23234.59 = 1.50309111 \times 10^8$ MeV

$$E_{TMZ(896\ \mu g/mL)\ in\ LDM} = \left[\ln\left(\ln\left[\frac{\ln 2}{10.82\times24\times60\times60}\right]\right)^2 - \ln\left(\ln\left[\frac{\ln 2}{5.12\times24\times60\times60}\right]\right)^2\right]$$

$\times 1 \times 10^5 \times 23234.59 = 2.53130851 \times 10^8$ MeV

In mice: LDM regimens of TMZ inhibit the tumor growth in U-87MG human glioblastoma model in a dose-dependent fashion after daily dosing for 25 days; the average tumor size of the control group grew from 10 mm^3 at the implantation to 120 mm^3 in 25 days (n = 15, P < 0.001) [19] with of 6.97 days. While, the average tumor size of the two treated groups by daily dose of 0.25 and 0.5 mg/kg for 25 days grew from 10 mm^3 to 130.1 and 58.77 mm^3 in 25 days (n = 15, P < 0.001) [19] with of 6.74 and 9.78 days respectively. TMZ doses of 0.25 and 0.5 mg/kg/d for 25 days in human (70 kg, 2.5L plasma) are equivalent to ([0.25 or 0.5] 25 70 mg/2.5L) 175 and 350 μg/mL respectively. Thus, from Equation 1 and 4, the energy yield by 175 and 350 μg/mL of TMZ in LDM regimens ($E_{TMZ\ in\ LDM}$) in tumor xenograft of intravenously transplanted 1×10^5 U-87MG human glioma cells were equivalent to:

$$E_{TMZ(175\ \mu g/mL)\ in\ LDM} = \left[\ln\left(\ln\left[\frac{\ln 2}{6.97\times24\times60\times60}\right]\right)^2 - \ln\left(\ln\left[\frac{\ln 2}{6.74\times24\times60\times60}\right]\right)^2\right]$$

$\times 1 \times 10^5 \times 23234.59 = 1.08680448 \times 10^7$ MeV

$$E_{TMZ(375\ \mu g/mL)\ in\ LDM} = \left[\ln\left(\ln\left[\frac{\ln 2}{9.78\times24\times60\times60}\right]\right)^2 - \ln\left(\ln\left[\frac{\ln 2}{6.97\times24\times60\times60}\right]\right)^2\right]$$

$\times 1 \times 10^5 \times 23234.59 = 1.13689785 \times 10^8$ MeV

Table 1 shows the energy yield by TMZ in the applied LDM regimens.

TMZ doses in μ g/mL	Energy yield by TMZ in LDM regimens
175	1.08680448×10^7 MeV
350	1.13689785×10^8 MeV
448	1.50309111×10^8 MeV
896	2.53130851×10^8 MeV

Table 1: shows the energy yield by TMZ in the applied LDM regimens.

The perfect correlation (r = 1) between the logarithms of TMZ doses of the applied LDM regimens and their corresponding energy derived in the presented *in-vivo* study shown in Figure 1 and Table 1 boost the confidence to establish the following efficient estimation model with a perfect fit (R^2= 1) shown in Figure 2 and expressed in Equation 5 to describe the energy yield by TMZ dose in LDM regimen. $E_{TMZ \text{ in LDM}} = 1.48340415 \times 10^8 \ln D - 7.55278448 \times 10^8$ MeV (Equation 5),

where D is the TMZ dose in g/mL applied in LDM regimen, $E_{TMZ \text{ in LDM}}$ is the corresponding energy yield of that dose in MeV.

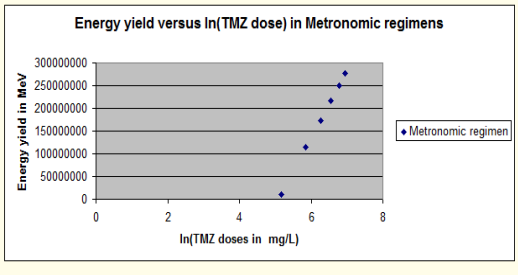

Figure 1: Shows a Scatter Plot Expressing Perfect Correlation (r = 1) between in (TMZ dose) and Energy Yield in Metronomic Regimens.

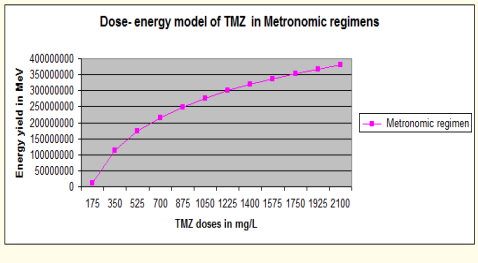

Figure 2: Shows the Increase of Energy Yield by Increasing TMZ doses in Metronomic Regimens Expressing the Non-Existence of MGMT-Resistance.

Figure 2 shows that the function of the energy yield by TMZ doses in metronomic regimes followed a logarithmic curve of no critical points (1st derivative can't be equivalent to zero) indicating the continuous increase of by the increase of $E_{TMZ \text{ in metronomic regimes}}$ the TMZ doses (D) in metronomic regimes up to 2 mg/kg/d. Accordingly, MGMT-resistance to TMZ doesn't exist in metronomic regimens of dose/day up to 2 mg/kg/d.

To evaluate the *in vivo* activity of TMZ in the standard regimens

In rats: Standard regimens of TMZ inhibit the tumor growth in C6/LacZ rat glioma orthotopic model. Kim., *et al*. Showed that the average tumor size of control group grew from 10 mm^3 at the implantation to 100 mm^3 (n = 10, P < 0.001) [19] in 17 days with doubling time (t_D) of 5.12 day. Thus, the average tumor size of the control group was 25.81 mm^3 at the beginning of TMZ therapy on day 7. While, the average tumor size of the treated group orally with 7 mg/kg TMZ for 5 days (between day 7 and 11 after intracranial implantation) to represent a tolerated standard regimen for TMZ therapy grew from 25.81 mm^3 to 75.8 mm^3 (n = 10, P < 0.001) [19] in 10 days from the beginning of TMZ therapy on day 7 to day 17 with of 7.17 days.

While Son., *et al* showed that the average tumor size of control group grew from 10 mm^3 at the implantation to 105 mm^3 (n = 14, P < 0.001) [20] in 17 days with doubling time ( ) of 5.01 days. Thus, the average tumor size of the control group was 27.65 mm^3 at the beginning of TMZ therapy on day 7.

While, the average tumor size of the treated group orally with 7.5 mg/kg TMZ for 5 days (between day 7 and 11 after intracranial implantation) to represent a tolerated standard regimen for TMZ therapy grew from 27.65 mm^3 to 78.16 mm^3 in 10 days from the beginning of TMZ therapy on day 7 to day 17 (n = 14, P < 0.001) [20] with of 6.67 days. TMZ doses of 7 and 7.5 mg/Kg/d for 5 days in human (70kg, 2.5L plasma) are equivalent to ([7 or 7.5] 5 70 mg/2.5L) 980 and 1050 µg/mL respectively. Thus, from Equation 1 and 4, the energy yield by 980 and 1050 µg/mL of TMZ in standard regimens ($E_{TMZ \text{ in Standard regimens}}$) in tumor xenograft of intravenously transplanted 1×10^5 C6/LacZ rat glioma cells were equivalent to:

$$E_{TMZ(980\ \mu g/mL) \text{ in Standard regimens}} = \left[\ln\left(\ln\left[\frac{\ln 2}{7.17 \times 24 \times 60 \times 60} \right] \right)^2 - \ln\left(\ln\left[\frac{\ln 2}{5.12 \times 24 \times 60 \times 60} \right] \right)^2 \right] \times 1 \times 10^5 \times 23234.59 = 7.89290040 \times 10^7 \text{ MeV}$$

$$E_{TMZ(1050\ \mu g/mL)\text{ in Standard regimens}} = \left[\ln\left(\ln\left[\frac{\ln 2}{6.67\times 24\times 60\times 60}\right]\right)^2 - \ln\left(\ln\left[\frac{\ln 2}{5.01\times 24\times 60\times 60}\right]\right)^2\right] \times 1 \times 10^5 \times 23234.59 = 9.85200300 \times 10^7\ MeV$$

In mice: Kim., *et al* showed that the standard regimens of TMZ inhibit the tumor growth in U-87MG human glioblastoma model in a dose-dependent fashion. The average tumor size of the control group grew from 10 mm^3 at the implantation to 120 mm^3 in 25 days (n = 15, P < 0.001) [19] with of 6.97 days.

Thus the average tumor size of the control group was 40.21 mm^3 at the beginning of TMZ therapy on day 14.While, the average tumor size of the treated group orally with 1.25 mg/kg TMZ for 5 days, (between day 14 and 18 after intracranial implantation) grew from 40.21 mm^3 to 70 mm^3 in 11 days (from day 14 to day 25) (n = 15, P < 0.001) [19] with of 13.75 days. The average tumor size of the other treated group orally with 2.5 mg/kg TMZ for 5 days, (between day 14 and 18 after intracranial implantation) shrunk from 40.21 mm^3 to 36.46 mm^3 in 11 days (n = 15, P < 0.001) [19]. From Equation 3, $\frac{40.21-36.46}{40.21}=\frac{40.21}{V_G-40.21}$ thus the volume of the equivalent virtual growing image that prevented from mitosis first of this shrinking tumor would be 471.2 mm^3 in 11 days with t_D of 3.1 days. TMZ doses of 1.25 and 2.5 mg/Kg/d for 5 days in human (70kg, 2.5L plasma) are equivalent to ([1.25 or 2.5] 5 70 mg/2.5L) 175 and 350 g/mL respectively. Thus, from Equation 1 and 4, the energy yield by 175 and 350 g/mL of TMZ in the standard regimens ($E_{TMZ\text{ in Standard regimens}}$) in tumor xenograft of intravenously transplanted U-87MG human glioma cells were equivalent to:

$$E_{TMZ(175\ \mu g/mL)\text{ in Standard regimen}} = \left[\ln\left(\ln\left[\frac{\ln 2}{13.75\times 24\times 60\times 60}\right]\right)^2 - \ln\left(\ln\left[\frac{\ln 2}{6.97\times 24\times 60\times 60}\right]\right)^2\right] \times 1 \times 10^5 \times 23234.59 = 2.25253411 \times 10^8\ MeV$$

$$E_{TMZ(375\ \mu g/mL)\text{ in Standard regimen}} = \left[\ln\left(\ln\left[\frac{\ln 2}{6.97\times 24\times 60\times 60}\right]\right)^2 - \ln\left(\ln\left[\frac{\ln 2}{3.1\times 24\times 60\times 60}\right]\right)^2\right] \times 1 \times 10^5 \times 23234.59 = 2.84224710 \times 10^8\ MeV$$

Table 2 shows the energy yield by TMZ in the applied standard regimens.

TMZ doses in μ g/mL	Energy yield by TMZ in standard regimens
175	2.25253411×10^8 MeV
350	2.84224710×10^8 MeV
980	7.89290040×10^7 MeV
1050	9.85200300×10^7 MeV

Table 2: Shows the energy yield by TMZ in the applied standard regimens.

Thus, from the given points in Table 2 the following efficient dose-energy model with a perfect fit (R^2=1) was established as shown in Figure 3 and expressed in Equation 6 to estimate the energy yield by TMZ dose in the standard regimens.

$E_{TMZ\text{ in Standard regimens}} = 1.93D^3 - 3728.06D^2 + 1880466.60\ D$ (Equation 6), where D is the TMZ dose in g/mL applied in standard regimen, $E_{TMZ\text{ in Standard regimens}}$ is the corresponding energy yield of that dose in MeV.

Figure 3 shows that the function of the energy yield by TMZ doses in standard regimes ($E_{TMZ\text{ in Standard regimens}}$) followed an inverted S-shaped curve having two critical points one of local maximum value (344.21 µg/mL, 2.84282515×10^8 MeV) and the other of local minimum value (943.55 g/mL, 7.6530142×10^7 MeV).

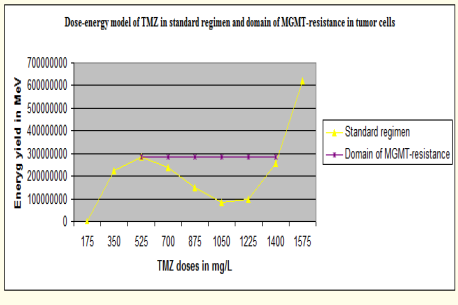

Figure 3: Shows Energy Yield by TMZ Doses and the Domain of MGMT-Resistance in Standard Regimens.

Accordingly, MGMT-resistance to TMZ was monitored at 344.21 g/mL TMZ in a standard regimen of 2.45 mg/kg bw/d for 5 days per 28 days/cycle (Figure 4), and then increased by the increase of the standard regimen dose and had a maximum value at 943.55 µg/mL TMZ in a standard regimen of 6.74 mg/kg bw/d for 5 days per 28 days/cycle (Figure 4). Afterwards, MGMT-resistance decreased gradually for MGMT depletion in tumor cells by the increase of the standard regimen dose until the complete suppression at 1243.22 µg/mL TMZ in a standard regimen of 8.88 mg/kg bw/d for 5 days per 28 days/cycle (Figure 4).

At this point the energy yield by 8.88 mg/kg bw/d for 5 days per 28 days/cycle was equivalent to that yield by2.45 mg/kg bw/d for 5 days per 28 days/cycle.

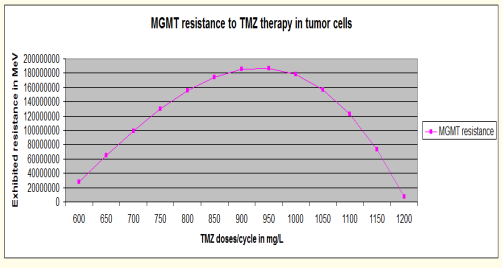

Figure 4: Shows the Increase in MGMT-Resistance to TMZ Therapy in Tumor Cells to its Maximum at 6.74 mg/kg bw/day (943.55 µG/Ml) and its Decrease by Increasing TMZ dose/day Until being Suppressed Due to MGMT Depletion in Tumor Cells.

Predicting the *in vivo* Activity of TMZ in Dose-Dense Regimen

Dose-dense regimen of TMZ inhibits the tumor growth in human melanoma UACC903 tumor model. The average tumor size of the control group grew from 100 mm^3 at the beginning of TMZ therapy at day 10 to 1200 mm^3 (n = 15, P = 0.0003) in 26 days at day 36 [22] with of 7.25 days. TMZ doses of 15 mg/kg/d for 5 days (on days 1, 3, 5, 7 and 9) in human (70kg, 2.5L plasma) are equivalent to (15 × 5 × 70 mg/2.5L) 2100 µg/mL. From Equation 6, the energy yield by 2100 M is 5.38196526 MeV. From Equation 2 and 4, the histologic grade (H_G) of the treated tumor xenograft of intravenously transplanted human melanoma UACC903 cells by 2100 M TMZ in dose-dense regimen of standard schedule of 15 mg/kg/d for 5 days in is supposed to be:

$$H_G = \ln\left(\ln\frac{\ln 2}{7.25\times24\times60\times60}\right)^2 \times 1\times10^6\times23234.59 + 5.38196526\times10^9 = 1.27058538\times10^{11}\ \text{MeV}.$$

Thus, from Equation 2, $H_{G/\text{hypoxic cell}} = 1.27058538\times10^{11}$ MeV / (1×10^6 cells) $=1.27058538\times10^5$ MeV/23234.59 =5.46850785 Emad. Accordingly, the treated human melanoma UACC903 tumor model would be predicted to grow with $(t_D)_{\text{Predicted}} = \ln 2 \times e^{\sqrt{e^{5.46850785\ \text{Emad}}}} = 3.37443341\times106$ sec= 39.05594229 days which is ~ 100%identical to that reported by Zhang L., *et al* about the actual growth of the treated human melanoma UACC903 tumor from 100 mm^3 to 158.6 mm^3 in 26 days (from day 10 to day 36) (n = 15, P = 0.0003) [22] with actual doubling time ($t_{D\ \text{Treated}}$) of $\left(\frac{\ln 2\times26}{\ln(158.6/100)}\right)$ 39.07466557 days. Thus, the actual response was 100% identical to the predicted one to provide a clear-cut criterion to accept the hypothesis of MGMT-resistance suppression by TMZ in dose-dense regimen and strengthen the confidence in predicting the therapeutic response by dose-energy model for energy yield by TMZ doses in standard regimens shown in Equation 6 and Figure 3.

Standard Regimen vs. Metronomic Regimen in TMZ Therapy

Figure 5 shows a comparison between efficacy of the standard regimens and the metronomic ones to differentiate between them; standard regimens of doses less than 4.37mg/kg bw/day for 5 days/ cycle of 28 days or more than 8.96 mg/kg bw/day for 5 days/ cycle of 28 days were more efficient than the metronomic ones of equivalent accumulative dose/day along the 28 days (Figure 5). While the metronomic regimens of doses more than 0.78 mg/kg bw/day for 28 days but less than 1.6 mg/kg bw/day for 28 days were more efficient than the standard ones of equivalent accumulative dose/day for 5 days /cycle of 28 days (Figure 5). In addition, by the end of the MGMT-resistance domain, the energy yield by both types of regimens were equivalent demonstrating same efficacy (Figure 5). These findings provide a clear-cut criterion to conclude that the ability of tumor cells to repair DNA in TMZ therapy is dependent mainly on dose/day that cells receive but not on the accumulated dose per the treatment cycle. Such ability was shown in the standard regimens starting from 2.45 mg/kg bw/day (344.21 g/mL) which had decreased the effectiveness of TMZ gradually by the increase of the received dose/day to its minimum at 6.74 mg/kg bw/d (943.55 g/mL). By increasing the received dose/day in the standard regimens more than 6.74 mg/kg bw/d gradually, the effectiveness of TMZ had been also increased gradually suppressing resistance by depleting MGMT in glioma cells. This led to support the suggestion of the extended dose-dense TMZ regimens for tumors of the advanced and recurrent stages, with the idea that they could potentially deplete MGMT in tumor cells by overwhelming the cells' ability to synthesize MGMT, which might enhance therapeutic activity.

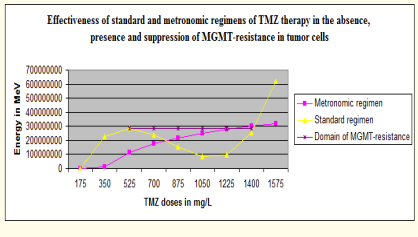

Figure 5: Shows the Energy Yield in Standard and Metronomic Regimens of TMZ Therapy in the Absence, Presence and Suppression of MGMT-Resistance in Tumor Cells.

Discussion

Despite recent advances in the antiangiogenic therapy with TMZ for primary and recurrent gliomas, survival remains poor. The role of MGMT to inhibit the response to TMZ effectively was shown in previous studies where the epigenetic inactivation of MGMT by hypermethylation of the promoter is accompanied by tumor response improvement and survival in gliomas patients [47-50]. But in the same time MGMT is consumed in the process of repairing TMZ-mediated DNA damage [51]. Current approach evaluated the resistance of MGMT expression in gliomas or melanoma to TMZ therapy. For the first time, dosing limits in metronomic or standard or extended dose-dense dosage regimen regarding the MGMT-resistance suppression were identified for all stages of primary or recurrent tumors to overcome resistance to TMZ therapy. The definition of metronomic chemotherapy has been changed several times in a manner which does not necessarily reflect the mechanism of action of the drug, but its pace and dose of administration. LDM regimen is applied on a frequent (daily, several times a week, or weekly) or continuous schedule with no extended interruptions, while dose-dense regimen has the typical schedule of the standard one with the increase in the accumulated dose per the treatment cycle. There are two oncologists' teams; one suggests that LDM regimen exerts its effects exclusively by killing the rapidly dividing endothelial cells in tumors, thus preventing angiogenesis [52,53], while the other supports the dose-dense TMZ regimens provided that MGMT would be depleted in tumors and thus MGMT-mediated resistance would be suppressed [51].

The clinical methodology for staging tumors were conducted as described in earlier studies to determine the energy yield by TMZ doses [35-40]. The consistent results confirm that MGMT-resistance is dependent mainly on the dose/day of the applied regimen. Its effect in tumor cells appears due to TMZ therapy from 2.45 mg/kg bw/day and increases gradually to its maximum at 6.74 mg/kg bw/day, and then decreases gradually by the increase of TMZ dose/day due to MGMT depletion in tumor cells -as shown in Figure 4 until being suppressed at 8.88mg/kg bw/day. Accordingly, the dosage limitations of each type of regimen were identified to optimize TMZ therapy for all stages of the disease as follows; from Figure 3, it becomes clear that standard TMZ regimens should be applied efficiently (overcome the effect the MGMT-resistance) on the primary tumors of early stages that is characterized by a relatively low histologic grade of administered dose/day doesn't exceed 4.37mg/kg bw/day for 5 days/ cycle of 28 days. While, efficiency of the standard regimens decreases due to the higher MGMT-resistance in tumor cells of moderate stages of administered dose/day lies between 4.37 and 8.96 mg/kg bw/day for 5 days/ cycle of 28 days as shown in Figure 3. Accordingly, LDM TMZ regimens would be more useful for those tumors of moderate stages as shown in Figure 5. Thus, for tumor relapse, the accumulated dose/cycle should be increased more than that of the primary one to overcome the acquired resistance by the first-line therapy regardless to type of therapy [28]. If the required accumulated dose/5 days for tumor relapse exceed 4.37 mg/kg bw/d but less than 8.96 mg/kg bw/d, a LDM TMZ regimen should be applied to avoid the higher values of MGMT-resistance and consequently optimize the TMZ dose. The administered dose of the standard regimen in such case would be distributed equally on all days of the LDM cycle without interruptions. With respect to tumors of the advanced stages, there are two options for choosing the optimal regimen depends on the tumor histologic grade and consequently on the required dose per cycle. If the required dose/5 days for the tumor of advanced stage exceeds 8.96 mg/kg bw/d, then the required dose/day will suppress the MGMT-resistance. Accordingly, a dose-dense regimen with standard schedule should be applied taking into account the maximum tolerated dose/day. But, if the required accumulated dose/5 days for the tumor of advanced stages surpasses the maximum tolerated dose/day, then a dose-dense regimen with metronomic regimen should be applied in which the required accumulated dose should be distributed equally on daily doses. The energy yield by TMZ doses in metronomic or standard regimens derived by the two-presented dose-energy models (Equation 5,6) was identified through in-vivo tumor models as conducted and described in earlier studies [25-27, 35- 40]. The perfect fit (= 1) of dose-energy model of TMZ therapy in metronomic regimen (Equation 5) strengthens the confidence to predict the therapeutic effect prior therapy as conducted and described in an earlier study [25-27,40]. Also, predicting the response of human melanoma UACC903 tumor model to 2100 M TMZ in dose-dense regimen of standard schedule of 15 mg/kg/d for 5 days was 100% identical to the actual response reported by Zhang L., *et al.* [22] to provide a clear-cut criterion about accuracy of dose-energy model of TMZ therapy in standard regimen (Equation 6). Predicting the response to TMZ requires to identify each of patient's histologic grade ($H_{G\ Control}$) and the estimated energy yield by TMZ dose derived by dose-energy model of either regimen as presented in section of results and analysis. Accordingly, to avoid treatment failure, patient's response to TMZ in either regimen should be predicted prior therapy to apply the optimal one for patient-specific H_G .

Conclusion

MGMT-resistance in TMZ therapy is dependent mainly on dose/day received by those cells. Its effect appears when applying TMZ standard regimens of 2.45 mg/kg bw/day for 5 days per a cycle of 28 days and increases by the increase dose/day till 6.74 mg/kg bw/d for 5 days per a cycle of 28 days. Thereafter, MGMT depletes in tumor cells gradually by the increase in the received dose/day until suppression of MGMT-resistance. Accordingly, our data suggest that standard TMZ regimen of administered dose less than 4.37 mg/kg bw/day for 5 days every 28 days is more efficient than the metronomic one of dose/day less than 0.78 mg/kg bw/day for 28 days in early stages of primary tumors. While the metronomic TMZ regimen of dose/day lies between 0.78 and 1.6 mg/kg bw/day for 28 days is more efficient than the standard one of administered dose lies between 4.37 and 8.96 mg/kg bw/day for 5 days every 28 days in the moderate stages of recurrent tumors to avoid the higher values of MGMT-resistance and consequently optimize the TMZ dose. Dose-dense TMZ regimens with standard schedule of dose/day higher than 8.96 mg/kg bw/ day for 5 days every 28 days or metronomic schedule of dose/day higher than 1.6 mg/kg bw/ day for 28 days suppress the process MGMT-mediated resistance in advanced stages of high-grade tumors by depleting MGMT in tumor cells.

Conflict of Interest

The Author declares no conflict of interest.

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