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Deleterious impact of a generic temozolomide formulation compared with brand-name product on the kinetic of platelet concentration and survival in newly diagnosed glioblastoma

Running title: Deleterious impact of generic temozolomide in glioblastoma

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ABSTRACT

Chemo-induced thrombocytopenia is a limiting toxicity among patients receiving temozolomide (TMZ) as first-line treatment for glioblastoma. We aimed to compare early platelet concentration kinetics, hematological safety profile and impact on survival following the initiation of either the brand-name or a generic TMZ formulation. A retrospective trial was conducted in patients suffering from newly diagnosed glioblastoma. Patients were treated with TMZ at 75 mg/m2 per day during six weeks, concomitantly with radiotherapy. Platelet concentration was collected each week. Primary endpoint was to perform a linear mixed effect model of platelet concentration kinetic over weeks. 147 patients were included: 96 received the brand-name TMZ and 51 received a generic TMZ formulation. Exposition to the generic was a significant variable that negatively influenced the platelet kinetics in the radiotherapy and concomitant TMZ phase, p=0.02. Grade ≥3 chemo-induced thrombocytopenia was more frequent in the generic group: 19.6% [95% CI 8.7-30.5%] *vs* 3.1% [0-6.6%], p=0.001. Exposition to the generic formulation of TMZ led to increase early treatment discontinuation due to TMZ-induced thrombocytopenia and was a worsening independent prognostic factor on overall survival: adjusted HR 1.83 [1.21-2.8], p=0.031. These data suggest that exposition to a generic formulation of TMZ *vs* the brand-name product is associated with higher early platelet decrease leading to clinically relevant impacts on treatment schedule in glioblastoma. Further prospective trials are needed to confirm these results.

Keywords

Chemo-induced thrombocytopenia, temozolomide, glioblastoma, generic drug, drug safety

ABBREVIATIONS

BSA, body surface area
CI, confident interval
EDTA, ethylenediaminetetraacetic acid
EORTC, European Organisation for Research and Treatment of Cancer
IMRT, intensity modulated radiation therapy
MRI, Magnetic resonance imaging
PFS, progression free survival
PPI, pump proton inhibitor
PTV, planning target volume
OS, overall survival
RPA, recursive positioning analysis
RT, radiotherapy
TMZ, temozolomide
WHO, World Health Organization

INTRODUCTION

Glioblastoma is the most frequent malignant brain tumor in adults [1]. Around 125.000-150.000 new cases of glioblastoma are diagnosed each year worldwide [2,3]. Surgery, when allowed by the tumor location, adjuvant radiotherapy (RT) and concomitant temozolomide (TMZ) followed by TMZ alone, also known as Stupp protocol, is the standard treatment for newly-diagnosed glioblastoma for patients younger than 70 and with good performance status [4]. Indeed, no alternative treatment regimen that does not include TMZ has resulted in a significant increase in overall survival [5]. The prognosis of glioblastoma remains poor with a one-year survival rate of 40-50% [6]. The correct exposition to TMZ along the entire treatment schedule is a key point for treatment outcome [7]. TMZ induces myelotoxicities, particularly thrombocytopenia [8]. All grade TMZ-induced thrombocytopenia occurs in one quarter to one third of patients in selected population of newly diagnosed glioblastoma patients. Severe TMZ-induced thrombocytopenia (<50G/L) are observed in 10%-20% of patients [9–13]. TMZ-induced thrombocytopenia leads to incomplete TMZ exposition by dose reduction, cycle delay or early discontinuation. The precise biological mechanisms underlying TMZ-induced platelet decrease and thrombocytopenia remain unclear. TMZ is an alkylating agent whose first evidence of effectiveness emerged in the late 1990s. Nowadays TMZ is widely used for glioblastoma patients and several formulations (i.e. brand-name TMZ or generic) are available. The impact of a generic chemotherapy formulation on the tolerance profile is discussed [14–17]. To our knowledge, the role of the generic formulation on the hematological safety profile of TMZ has never been studied. We recently highlighted that one third of patients had a significant decrease of platelets during the concomitant RT-TMZ phase, independently from their clinical characteristics [18]. Platelet decrease during the RT-TMZ phase was strongly correlated to TMZ-induced thrombocytopenia in the maintenance phase. We hypothesize that TMZ formulation (brand-name versus generic) may have contributed to the early platelet decrease and subsequently to the occurrence of thrombocytopenia. Thus, we conducted a retrospective analysis to investigate the impact of the TMZ formulation on hematological safety profile and survival.

The main objective of this study was to investigate the impact of TMZ formulation on platelets kinetic in the RT-TMZ phase and on survival in patients suffering from newly diagnosed glioblastoma.

MATERIALS AND METHODS

Study design and patient population

A retrospective monocentric study on previously published population was conducted. Inclusion and non-inclusion criteria were previously described [18]. In brief, patients were suffering from newly diagnosed glioblastoma according to 2016 WHO classification. According to the Stupp protocol, TMZ was administrated orally at 75 mg/m2 of body surface area (BSA) every day for six weeks during the RT-TMZ phase [4]; the maximum daily dose was 150 mg. In the RT-TMZ phase, TMZ was weekly and centrally delivered by our institution. Study population was divided into two groups: first group was composed by patients treated from January 2009 to July 2013 who received the brand-name TMZ (bnTMZ, Temodal®, Merck Sharp & Dohme Ltd, Hoddesdon, Hertfordshire, United Kingdom) and the second group consisted in patients who received a generic TMZ formulation (genTMZ, Temozolomide Sun®, Sun Pharmaceutical Industries Ltd, Bombay, India) from August 2013 to December 2014. In order to ensure the absence of a period-effect in the bnTMZ group, we divided the inclusion period in two equivalent periods of time and compared the characteristics of hematological toxicities and platelet decrease between these two groups.

This study was conducted in accordance with the Declaration of Helsinki and was approved by the institutional review board of Observational Health Research of our institution (number 1707B).

Measurements of the platelet concentration and modelization of their kinetic

Platelet concentrations were evaluated at the beginning of each out of six weeks during the RT-TMZ phase. Blood samples were collected from peripheral venous blood using 4mL EDTA tubes. Platelet concentrations were estimated by the electric impedance and optical methods using XN-1000 hematology analyzer (Sysmex Corporation®, Kobe, Japan). First, platelet count was measured by the impedance method; in case of platelet abnormal distribution a second analysis with the optical method was automatically carried out. In case of final platelet count lower than 100G/L or higher than 700G/L, a blood smear was performed to visually control platelet count.

Modelization of Platelet Kinetic and Statistical Analyses

Statistical analyses were performed using the R software (R version 3.5.1, 2018, Vienna, Austria). Parametric distribution of the data was assessed by Shapiro test. All normally distributed continuous data were expressed as mean and 95% confident interval [CI]. Categorical variables were presented as absolute and relative frequencies. Data were compared using Student's unpaired t-test for continuous variables and the Fisher exact test or Pearson's chi-squared test was used for categorical variables, as appropriate.

Model construction

The studied variable was the platelet concentration. The explanatory variables were: time (week of treatment), formulation of TMZ (bnTMZ versus genTMZ), age, gender, BSA, creatinine clearance, tumor volume defined as the planning target volume (PTV) according to EORTC guidelines [19] and concomitant use of glucocorticoids and pump proton inhibitor (PPI). In addition, although not directly involved in TMZ metabolisation CYP450 inducers, inhibitors and substrates were included in the exploratory variables.

First the correlation between all the variables of the model has been explored. The R package ade4 and its function dudi.mix was used [20]. Dudi.mix function provides a graphical representation of the correlation between both qualitative and quantitative variables. dudi.mix provides correlation for each modality of categorical variables. To do so, dudi.mix assigns a coefficient to each variable (Eigenvalues) and selects the two categories of variables that have the most correlation with all the others. Once selected, these two modalities serve as reference coordinates for the graphical representation. Then the variables are represented in a two-dimensions plot: two vectors pointing in the same direction represent a strong positive correlation between two variables; conversely, two vectors pointing at 180° represent a strong negative correlation. Two vectors pointing at right angle means no correlation.

Second a linear mixed-effect model was performed to assess the association between platelet concentration and all explanatory variables. Univariate linear regression for each variable was performed using the *lmer* function in order to investigate their relation with the platelet concentration. Final linear mixed-effect model was conducted with the *lmer* function (package lmerTest, version 3.1-1). Accordingly, the model used was a mixed-effect model and the variables were correlated to each other and particularly to the time. To perform a multivariate analysis of mixed variables, an ordination of both quantitative and qualitative variables was conducted. Exploratory variables included in the final multivariate model are all the variables with an alpha risk threshold lower than or equal to 10% in univariate analysis. The hypothesis of linear platelet decrease during RT-TMZ phase was stated. Ten linear models were generated among the possibilities of construction between fixed and random parameters. The selected final model was the one with the lowest AIC.

TMZ-induced thrombocytopenia was collected during the RT-TMZ phase. Finally, an exploratory comparison of thrombocytopenia between bnTMZ and genTMZ group was performed during the TMZ maintenance phase.

Survival analysis

Exploratory survival analysis was performed in each set of patients. Exposure to the genTMZ versus the bnTMZ was investigated as risk factor for overall survival (OS) and progression free survival (PFS). OS was calculated in months from the date of histological diagnosis to the date of death for any reason. PFS was calculated in months from the date of histological diagnosis to the date of progression on MRI defined by the Response Assessment in Neuro Oncology criteria [21]. Prognostic impacts of exposure to each TMZ formulation was estimated after adjustment on Karnofsky performance status index, age, initial steroid exposure at the time of diagnosis and extend of initial surgical resection. The log-rank test investigated OS and PFS differences among groups. Survival analyzes were performed using the Cox regression model after testing proportional hazard assumption, supplementary material. In univariate analyses alpha risk threshold was set at 10% to select variables for multivariate analyzes; alpha risk threshold was set at 5% in the final Cox regression model.

RESULTS

Patients' characteristics and TMZ-induced hematological toxicities

One hundred and forty-seven patients were included. Patients' demographics and characteristics are presented in Table I. Ninety-six patients received the bnTMZ and 51 patients received genTMZ. Patients receiving genTMZ were significantly older as compared with bnTMZ group (p=0.008). Patients under 50 years of age were more likely in the bnTMZ group: 35% versus 20% (p=0.046). As a consequence, genTMZ group had a higher proportion of class IV recursive positioning analysis (RPA): 51% versus 32% (p=0.014). Proportion of intensity modulated radiation therapy (IMRT) was significantly lower in the bnTMZ group: 12 patients/96 (13%) versus 46 patients/51 (90%) in the genTMZ group (p<0.001). All patients completed the planned radiotherapy phase. In the studied population, twenty-one patients (14%) stopped TMZ before the end of the RT due to TMZ-induced thrombocytopenia lower than 100G/L. Proportions of all grades thrombocytopenia and severe thrombocytopenia were significantly higher in the genTMZ group, both during the RT-TMZ phase and the maintenance phase, Table II. Eight patients (5%) were hospitalized during the RT-TMZ phase for platelet transfusions: three patients (3%) in the bnTMZ group and five patients in the genTMZ group (10%) (p=0.13). No clinically significant bleeding was reported. Six patients (4%) never started the TMZ maintenance phase due to prolonged thrombocytopenia: one patient in the bnTMZ group and five patients in the genTMZ group (p=0.025). One severe pulmonary infection was observed in the genTMZ group. Two patients suffered from grade 3 anemia in the genTMZ group versus none in the bnTMZ group, (p=0.11). Baseline clinical characteristics of patients experiencing thrombocytopenia <50G/L are detailed in Supplementary Table I. No difference was observed in the occurrence of grade 3-4 toxicities on leukocyte lines in the RT-TMZ phase: 7.3% of lymphopenia in the bnTMZ group versus 9.8% in the genTMZ group (p=0.75) and 1% of neutropenia versus 5.9% (p=0.12). 88 patients (60%) received antiepileptic treatment with levetiracetam during the RT-TMZ phase. No other antiepileptic molecule was used during this period. Platelet decay was not influenced by exposure to levetiracetam: mean decrease of -21.5% in the exposed group versus -22.5% in the unexposed group (p=0.86). No particular dietary regimes have been reported, especially any ketogenic diet.

During the TMZ maintenance phase, the exposition to the drug was lower in the genTMZ group. Accordingly, mean number of maintenance cycles was 4.6 cycles [3.4-5.8] in the genTMZ group versus 8 cycles [7-9.1] in the bnTMZ group (p<0.001). Likewise, mean TMZ dose per day per cycle was 165 mg/m2 [161-169] in the genTMZ group versus 182 mg/m2 [179-182] in the bnTMZ group (p<0.001).

No significant difference was observed between genTMZ and bnTMZ regarding excipients (data not show).

Correlations between TMZ formulation and clinical characteristics

Absolute platelet concentration was independent of exposure to bnTMZ or genTMZ. genTMZ exposure was correlated to absolute PTV, mainly due to high volumes (PTV >372cm3), supplementary Figure S1. Conversely, exposure to bnTMZ correlated with low volumes of PTV (<175cm3). In order to reduce the effect of these correlated variables, only individuals with PTV lower than the mean plus three times the standard deviation (665cm3) were selected in the final model. Results of the Grubbs test suggested to consider four individuals as outliers (PTV values 966, 849, 751 and 674 cm3, p= 0.0098).

Platelets concentration in the RT-TMZ phase

Platelets concentrations decreased in both groups during the RT-TMZ phase: mean platelet concentration at baseline 257G/L [247-267] versus 189G/L [174-204] at week 6 ($p<0.001$). Mean platelets concentrations were significantly lower in the genTMZ group during the 4th to 6th week period, Figure 1A. Considering the nadir of platelets count, the average proportion of platelet concentration was also significantly lower in the genTMZ group when compared to the baseline value: mean of 60%, [52-68%] versus 79% [74-84%] in the bnTMZ group ($p<0.001$), Figure 1B. Linear mixed-effect model was performed on 145 patients: 96 patients in the bnTMZ group and 49 patients in the genTMZ group. Univariate analyses demonstrated a significant correlation between platelet concentration kinetic and time (week), BSA, PTV, prednisone daily dose, CYP450 substrate exposition, PPI exposition, age and TMZ formulation. However, final linear mixed-effect model showed that exposition to genTMZ was a significant and independent variable that influences the platelet decrease ($p=0.021$), Table III. Normality distribution of residuals was checked and non-linearity, or unequal error variance was not detected (Supplementary Figure S2). In addition, the decrease of platelet concentration was associated to PTV values, BSA as well as corticosteroids doses, Table III. Conversely, PPI intake was associated with platelet increase. Although age was not a statistically-relevant variable in multivariate model, when increasing it was associated to a trend toward a platelet decrease.

Considering that the inclusion period of patients exposed to bnTMZ was longer than that of patients exposed to genTMZ (respectively 53 months vs 19 months), a potential "period effect" was investigated in the bnTMZ group. The inclusion period for bnTMZ patients was divided into two periods: period 1 from April 2009 to July 2013 (n=49) and period 2 from August 2013 to January 2015 (n=47). No period-related difference was statistically identified in both the proportion of severe hematologic toxicities (8.1% in period 1 vs 10.6% in period 2, $p=0.68$) and platelet nadir during RT-TMZ phase (79% vs 77%, $p=0.79$).

Survival analyses

Median follow-up was 45 months in the whole population (range 13-85 months). Median OS was 18 months [17-21] and median PFS was 10 months [9-12] in the entire cohort.

Exposition to genTMZ was associated to worse OS: median OS was 20 months in the bnTMZ group versus 17 months in the genTMZ group, HR 1.83 [1.21-2.8, $p=0.004$], Figure 2 and Figure 3. 2-years OS was significantly lower in the genTMZ group: 9.8% [1.6-18%] versus 32.2% [22.7-41.3%] in the bnTMZ group ($p=0.003$). In univariate analysis, median PFS was significantly lower in the genTMZ: 9 months versus 11 months ($p=0.031$). This difference on PFS was not significant in multivariate analyses, Figure 3. As expected, age was associated to prognosis both on OS and PFS. In multivariate analyses, Karnosky Index, extent of resection and corticoid exposition did not influence OS or PFS, probably due to a lack of power.

DISCUSSION

The exposure to a generic formulation of TMZ influences the decay of platelets and is associated to an increased risk of thrombocytopenia in the RT-TMZ phase. Our work is the first to focus on TMZ-induced thrombocytopenia in chemotherapy naive and homogeneous population of newly-diagnosed glioblastoma patients.

Concerning the methodology used, our results were obtained in a large cohort of homogeneously treated patients in a reference center with a centralized collection of biological parameters. In addition, the proportions of thrombocytopenia ≤100 G/L (19%) and thrombocytopenia <50 G/L (7.3%) in the group exposed to bnTMZ are comparable to those found in the literature [4,9,10,12] suggesting that our whole study cohort reflects daily practice population. In this context, patients with genTMZ have a higher proportion of thrombocytopenia ≤100 G/L (33%) and thrombocytopenia <50 G/L (19.6%). This clinically relevant difference could be explained by the fact that clinical tests and marketing authorization dossiers are simplified for generic medicines compared to the brand-name product. The required bioequivalence must be between 80% and 125% of both AUC and Cmax of the brand-name product [22]. Nevertheless, a review of bioequivalence trials shows Cmax and AUC variations of less than 10% for 98% of the generics studied [23]. However, an increase in Cmax and AUC could lead to an over risk of toxicities that would not be identified in low power cohort bioequivalence trials. Differences in safety profile between brand-name and generic formulation of chemotherapy have been explored in previous studies. Oike et al showed that grade ≥3 leukopenia were more frequent (p=0.034) in a population of patients receiving generic cisplatine concomitant to radiotherapy for uterine cervical cancer [14]. Chambord et al also found a higher proportion of ifosfamide-induced encephalopathy in the group of patients receiving generic formulation vs brand-name product: 10.2% vs 1.9%, respectively [24]. In contrast, regarding differences in the occurrence of all grade renal toxicities in the cisplatine generic formulation group the results remain controversial [15,16,25]. These results suggest that the impact of formulation may differ according to the product and to affected organ.

Furthermore, errors in drug intake have been previously described in the RT-TMZ phase [26] and may have occurred more frequently in the genTMZ group. In our population medication errors were limited by centralized TMZ dispensation and dispensing or prescription errors were not reported. Unfortunately, the certainty of TMZ compliance has not been formally confirmed by the lack of collection of empty blisters. Moreover the weekly maximal delivered dose of TMZ was 525 mg/m2 (75 mg/m2/day for seven days) in both bnTMZ and genTMZ group and remains lower than the 5-days schedule at 150 mg/m2/day currently used in the maintenance phase. Thus, the risk of acute dose-dependent hematological toxicities in the RT-TMZ phase resulting from an overexposure to TMZ was very low and similar in both groups. Although no information was available concerning dose concentration relationship.

Due to the retrospective design of this study, potential biases can be identified. Regarding baseline characteristics significant differences between the two groups were observed on age, proportion of IMRT and PTV. Although the absolute difference between the ages in the studied groups was statistically significant, it was not considered as clinically relevant and did not appear in the final multivariate model. Two studies did not identify age as a risk factor for TMZ-induced myelotoxicities (8,27). Due to the evolution of radiation therapy over the last decade, 90% of the patients in the genTMZ group were treated using the IMRT versus 13% in the bnTMZ group. The incidence of acute hematologic toxicity was previously described to be equivalent for patients undergoing IMRT versus three-dimensional conformal radiotherapy in gynecologic cancer [28–30]. More recently, Byun et al have even

demonstrated that IMRT for patients receiving chemoradiation for glioblastoma is associated with a lower proportion of acute severe lymphopenia [31]. Mean PTV was significantly higher in the genTMZ group (346.6 cm3 versus 258.8 cm3, p=0.002). This may have impacted platelet decrease in the genTMZ group, as previously described for lymphocytes. Indeed, PTV remains a significant parameter in the final multivariate model, independently from the considered TMZ formulation. PTV may have participated to the results but did not explain the whole difference between both groups. Of note, since thrombocytopenia evaluation was performed centrally, a potential bias of under evaluation of this event is unlikely. Finally, according to the different period of recruitment between the two groups, one would expect a possible period-effect to explain such a difference but no time-related difference was observed within the group of patients treated with bnTMZ.

Three parameters influenced platelet kinetic in the RT-TMZ phase in the final multivariate model: genTMZ exposition, BSA and concomitant medication with PPI. The impact of high values of BSA on safety profile is probably due to misestimating body composition metrics [32] leading to a higher dose administration than those justified by lean body mass [33]. In accordance, fat mass index and skeletal muscle parameters are better predictors of chemotherapy related toxicities [34]. Whatever the impact of BSA is independent from TMZ formulation. Concomitant medication with PPI appeared to positively influence platelets kinetics in the RT-TMZ phase. To our knowledge, no study investigated the plasma concentration of TMZ or its active metabolite (3-methyl-(triazen-1-yl)-imidazole-4-carboxamide, MTIC) when co-administered with PPI. TMZ hydrolyses to MTIC in plasma at physiological pH [35]. The variation of gastric pH due to pump proton inhibitor may have decreased the bioavailability of TMZ. This mechanism remains speculative and cannot explain the higher decrease of platelet concentration observed in the genTMZ.

Our study also shows for the first time that generic chemotherapy exposition could have a worsening impact on survival. One explanation is the occurrence of early severe hematological toxicities leading to overall under-exposure of chemotherapy in the genTMZ group. Once again, the generic marketing authorization does not require confirmation of clinical efficiency but only biological equivalence with the limitations previously discussed. Prognostic impact of under-exposure due to more frequent toxicities may not be significant in the small cohorts of bioequivalence trials. Nevertheless, worsening survival impacts of generic formulation need to be explored in larger cohort and dedicated prospective trial.

The proportion of other severe hematologic toxicities was not found statistically different between patients receiving the two formulations. Those results are probably due to a lack of power given the low occurrence of grade 3-4 anemia or neutropenia in larger population: 1.3% [10] and 4% [11], respectively. Nausea, asthenia and headaches are the most common TMZ-related adverse events reported in clinical trials. Unfortunately, those adverse events did not have a uniform data collection in our study. It would be of interest to prospectively collect them in further studies.

Finally, our model did not integrate pharmacogenetics-related predisposition to TMZ-induced acute myelotoxicity [27,36,37]. Further prospective study is planned at our institution to investigate a possible role of *MGMT* polymorphisms in TMZ-induced thrombocytopenia (GLIOPLAK trial, clinicaltrials.gov, NCT02617745). Furthermore, the hypothesis of linear platelet decrease was stated and, therefore, linear mixed-effect model was applied. We found that platelet kinetic in the RT-TMZ phase could actually follow a non-linear decrease: initial "steady-state" phase for 3-4 weeks followed by rapid fall. We did not explore the hypothesis of non-linear decrease

because. Indeed, regarding the significance of TMZ formulation in final linear model and the impact of generic formulation both on platelet absolute value and occurrence of early thrombocytopenia, it is likely that the variable TMZ formulation would have remained significant in non-linear mixed model. Nevertheless, the non-linearity hypothesis should be properly investigated and will be explored in ongoing GLIOPLAK trial.

As a conclusion, this study highlights a differential impact on safety profile, treatment schedule and survival based on TMZ formulation as first-line treatment of glioblastoma. The studied generic was associated with decreased platelet concentration and survival in newly-diagnosed glioblastoma patients. The mechanistic explanation remains unsolved and further prospective trials could help to understand such a differential effect.

AUTHORIZATION

Institutional Review Board approved the study protocol (1707B).

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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LEGENDS TO FIGURES

Figure 1. Platelet concentrations during the RT-TMZ phase.

(A) Platelet concentrations decrease with time in the two TMZ groups but with higher magnitude in the generic TMZ group. (B) The maximum decrease (%) of platelet concentration per patient was higher in the generic TMZ group vs the brand-name TMZ group. The reference platelet concentration was the concentration at week 0 and the comparison value was the platelet concentration at the time of the maximum effect in the RT-TMZ phase. Red points represent the means of each group and error bars represent their 95% confidence interval.

bnTME, brand-name temozolomide ; genTMZ, generic temozolomide; ns: non-significant, *: $p<0.05$; **: $p<0.01$, ***: $p<0.0001$

Figure 2. Kaplan-Meier curves.

Progression-free survival (A) and overall survival (B) curves as functions of TMZ formulation. Solid vertical lines represent censored data.

bnTMZ, brand-name temozolomide; genTMZ, generic temozolomide.

Figure 3. Forest Plot for Cox Proportional Hazards Model.

Representation of the hazard ratio of the logistic regression model in multivariate analysis of overall survival (A) and progression-free survival (B).

**: $p<0.01$

SUPPLEMENTARY FIGURES

Supplementary figure S1. Correlation between variables of the linear mixed-effect model.

The histogram on the top left summarizes the contribution of each variable to the final correlation plot using a coefficient called Eigenvalue. The two first black bins are the main variables referring to the maximum information ('CYP450 inhibitor exposure' and 'PTV <175cm3'). These two variables secondly serve as reference axes in the main plot. The two-dimensions plot gives the relations between variables (both factors and quantitative variables) materialized with a scatter plot and vectors. The positive (same vector direction) or negative (opposite vector direction) correlation or no correlation (right angle vector direction) between the three interest variables ('generic TMZ', 'brand-name TMZ' and 'platelet') and the others variables are represented.

CYP, cytochrome; induc, inducer; inhib, inhibitor; PTV, planning target volume; TMZ, temozolomide.

Supplementary figure S2. Model accuracy.

Graph of the assumption of the normal distribution of residual, Q-Q plot (A) and graph with residuals versus fitted values (B).

Table I. Population characteristics at baseline

		Entire cohort n = 147	Brand-name TMZ n = 96	Generic TMZ n = 51	p
Age (years), mean [95% CI]		55.1 [53-57.2]	53.2 [50.5-55.9]	58.7 [55.7-61.6]	0.008
Gender n (%)	Female	56 (38%)	38 (40%)	18 (35%)	0.61
	Male	91 (62%)	58 (60%)	33 (65%)	
Body surface area (m2), mean [95% CI]		1.9 [1.86-1.93]	1.89 [1.84-1.94]	1.91 [1.85-1.96]	0.67
Creatinine clearance (ml/min/1.73m2), mean [95% CI]		89.7 [85-94.4]	90.4 [84.1-96.6]	88.5 [81.3-95.6]	0.69
PTV (cm3) mean, [95% CI]		289.3 [263.3-315.3]	258.8 [228.5-289.1]	346.6 [300.6-392.5]	0.002
RPA stage, n (%)	III	40 (27%)	30 (31%)	10 (20%)	0.048
	IV	58 (39%)	31 (32%)	27 (51%)	
	V or VI	49 (34%)	35 (37%)	14 (29%)	
Concomitant therapies					
Glucocorticoids, n (%)		90 (61%)	57 (59%)	33 (65%)	0.53
Prednisone-equivalent daily dose (mg) mean, [95% CI]		20.1 [16.1-24.5]	20 [14.2-25.7]	20.9 [15.3-26.5]	0.81
CYP450 inducer, n (%)		8 (5%)	4 (4%)	4 (8%)	0.008
CYP450 inhibitor, n (%)		2 (1%)	1 (1%)	1 (2%)	1
CYP450 substrate, n (%)		102 (69%)	64 (67%)	38 (75%)	0.45
Pump Proton Inhibitor, n (%)		58 (39%)	33 (34%)	25 (49%)	0.08

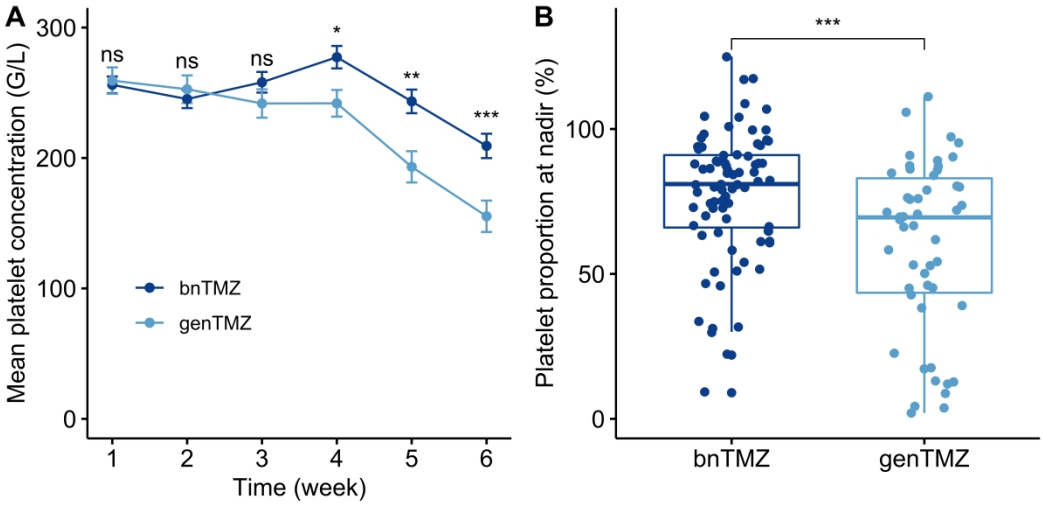
Table II. Temozolomide-induced thrombocytopenia during the RT-TMZ phase and the TMZ maintenance phase

	Brand-name TMZ n=96	Generic TMZ n=51	p
RT-TMZ phase			
Thrombocytopenia ≤100 G/L	9 (9.4%)	12 (23.5%)	0.02
Thrombocytopenia <50 G/L	3 (3.1%)	10 (19.6%)	0.001
RT-TMZ phase + TMZ maintenance phase			
Thrombocytopenia ≤100 G/L	18 (19%)	17 (33%)	0.048
Thrombocytopenia <50 G/L	7 (7.3%)	10 (19.6%)	0.03

Table III. Univariate linear regression and multivariate mixed-effects regression analyzes investigating the influence of covariates on platelet concentration during the RT-TMZ phase.

Covariate	Category	Univariate analysis		Multivariate analysis†	
		β coefficient ±S.D	P	Adjusted β coefficient ±S .D.	P
Week	Intercept	277.9 ±7.54	< 2e-16	499.2 ±38.6	< 2e-16
	Per week increase	-10.2 ±1.7	2.9e-08	-11.45±1.5	4.20e-13
Age	Intercept	274.39 ±24.44	<3.1e-14		
	Per year increase	-0.61	0.1	-7.8e-04	0.56498
Gender	Intercept	249.2 ±9.9	2e-16		
	Male	-13.8 ±11.95	0.251	-	-
Body Surface Area	Intercept	342.26 ±47.11	3.5e-11		
	Per m2 increase	-53.5 ±24.4	0.0307	-69.86±15.1	4.50e-06
Planning Target Volume	Intercept	283.51 ±10.5	2e-16		
	Per cm3 increase	-0.15 ±0.03	2.7e-05	-0.08±0.02	8.36e-06
Creatinine clearance	Intercept	247.71 ±19.04	9.9e-13		
	Per ml/min/1.73m2 increase	-0.079 ±0.21	0.712	-	-
Prednisone equivalent daily dose	Intercept	261.46 ±6.74	2e-16		
	Per mg increase	-1.05 ±0.24	0.0001	-0.79±0.12	5.63e-11
CYP450 inhibitor	Intercept	240.92 ±5.8	2e-16		
	Yes vs No	-4.6 ±24.49	0.858	-	-
CYP450 inductor	Intercept	240.99 ±5.66	2e-16		
	Yes vs No	-20.9 ±65.28	0.08	-35.42±23.45	0.1314
CYP450 substrate	Intercept	271.63 ±9.2	2e-16		
	Yes vs No	-44.9 ±11.3	0.000141	-16.06±7.3	0.0293
Pump proton inhibitor	Intercept	255.37 ±7.1	2e-16		
	Yes vs No	-36.45 ±11.1	0.00132	2.46± 6.7	0.7160
Molecule	Intercept	249.74 ±7.26	2e-16		
	Generic TMZ versus brand-name TMZ	-25.96 ±11.05	0.0204	7.21±5.9	0.03813

† Exploratory variables included in the multivariate analysis with alpha risk threshold ≤10%



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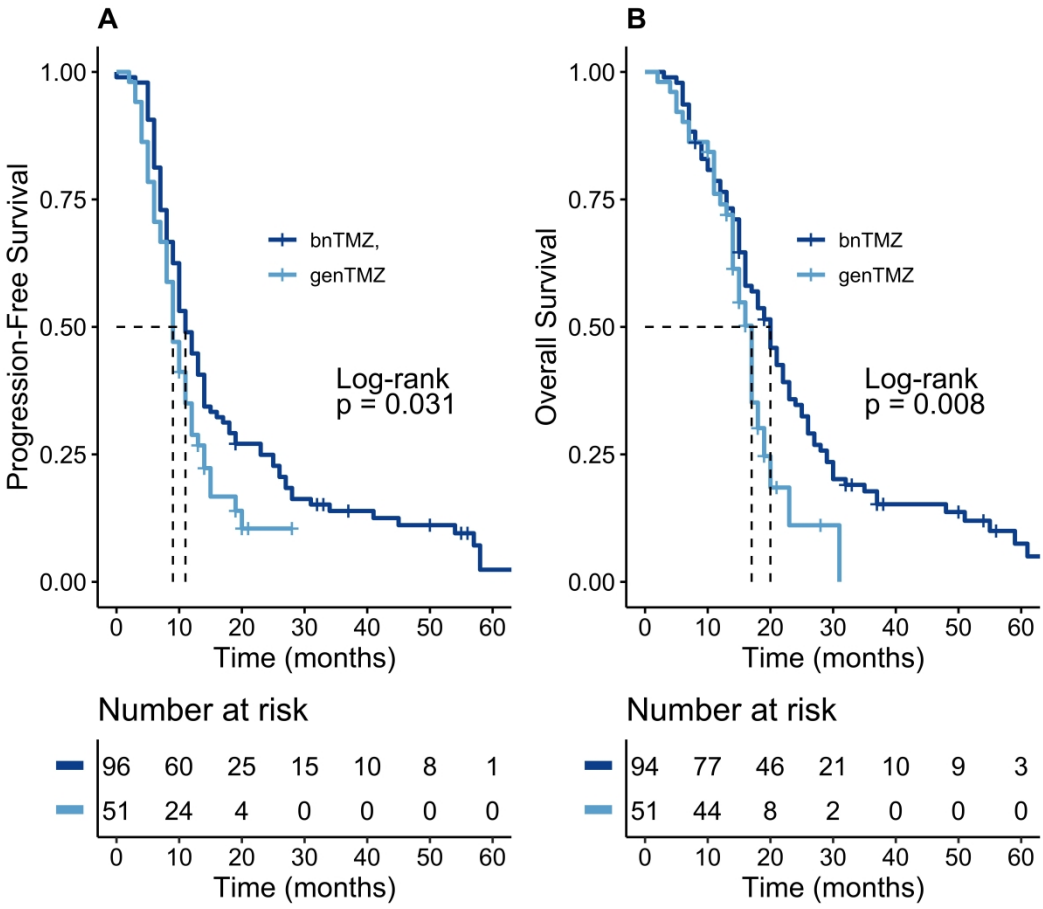

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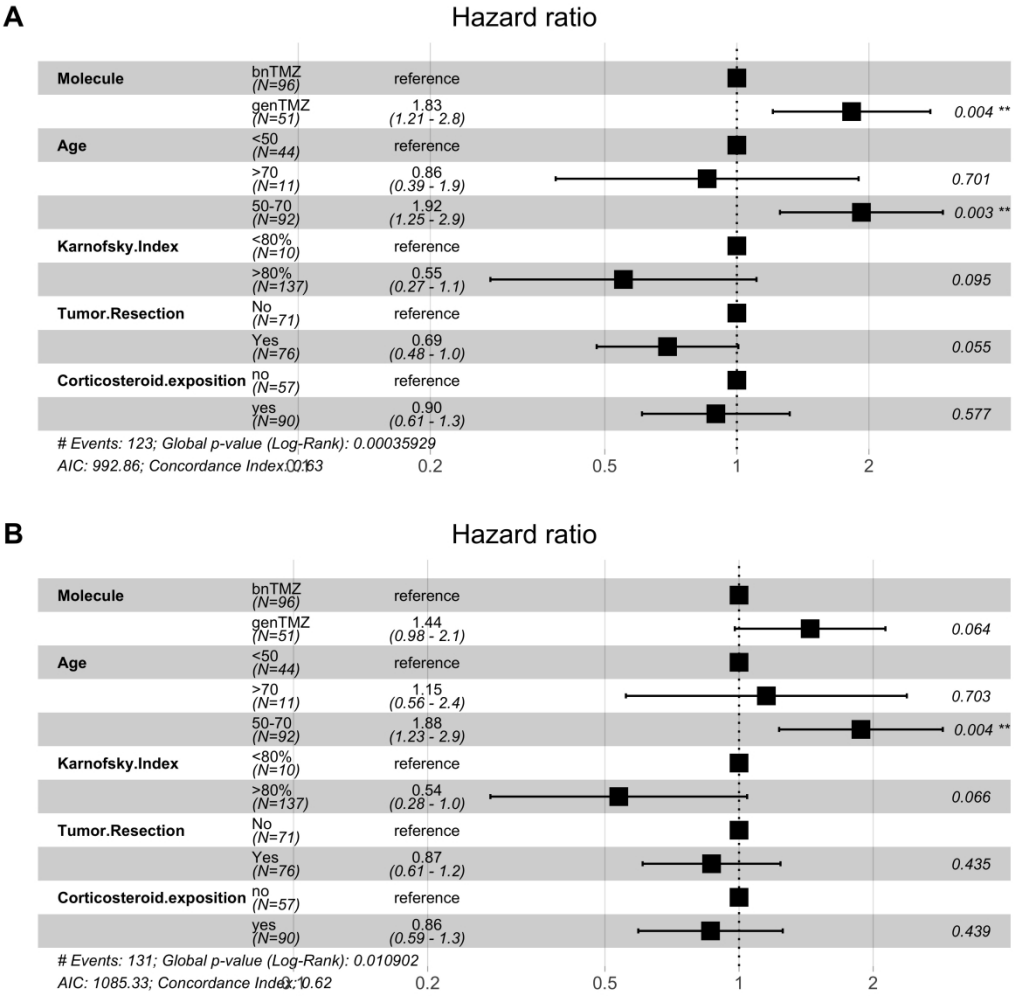

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