TITLE: CLINICAL TRIAL PHASE IIB RANDOMIZED, MULTICENTER, OF CONTINUATION OR NON-CONTINUATION WITH 6 CYCLES OF TEMOZOLOMIDE AFTER THE FIRST 6 CYCLES OF STANDARD FIRST-LINE TREATMENT IN PATIENTS WITH GLIOBLASTOMA.

Statistic Plan MAY 2018

STATISTICAL CONSIDERATIONS
Sample size

The sample size is calculated based on the hypothesis defined in the main objective of not exceeding Progression-free survival at 6 months in the continuation of 6 to 12 months of treatment, between methylated and unmethylated patients.

Based on data from the EORTC-NCIC study (Stupp et al, 2005, 2009 ref 3), a probable PFS quality of 0.593 in methylated, 0.285 in unmethylated. 95% confidence is assumed, 80% of statistical power and is calculated based on a ratio of 1: 1.5 to have more methylated patients.
With a maximum loss of 10%, the sample must be 32 non-methylated patients and 48 methylated patients (total n = 80) in the TMZ treatment group. In order to respond to the comparison objective, the same sample is established in a group of untreated patients.
This would lead us to have a sample of 64 patients unmethylated and 96 methylated for a total of 160 patients included. (n = 160).
Considering that patients with residual disease can potentially benefit compared to those who do not have it, this variable is established as a randomization factor, and taking into account. Reports that 60% of residual disease is expected in methylated patients and 40% in non-methylated patients, 4 randomization groups are established so that of the 32 non-methylated 13 are with residual disease and of the 48 methylated 23 are with residual disease.

Study variables
Primary efficacy variable

The main study variable to determine the differences between the two treatment groups will be progression-free survival at 6 months. This variable will be evaluated in patients with glioblastoma that they have already received 6 cycles of temozolomide (adjuvant) without progressing and are randomized to continue with 6 additional cycles of temozolomide or stop treatment, from the date of randomization up to date of the progression defined according to the RANO criteria. Patients who are living and without evidence of progression at this time will be considered as successes and those who have progressed or died at this time will be considered as treatment failures. The diagnosis of progression must be based on the RANO criteria.

Disease progression is defined as:
1. radiological worsening (increase in contrast enhancement area or appearance of lesions new), that is, RANO progression criteria.
2. significant worsening in FLAIR along with irreversible neurological impairment.

3. irreversible neurological deterioration even in the absence of radiological deterioration.
4. continued increase in corticosteroids (> 2 weeks) to prevent neurological deterioration.

Secondary variables
The two treatment arms will be determined and compared:

Clinical, biological and demographic data: (sex, age, type of surgery, initial MMS, initial Bartel index, presence of neurological symptoms (mild, moderate, important), bevacizumab or not in the treatment of
first line, treatment characteristics, second line treatments.

Safety / Toxicity Profile: Type, incidence, severity, frequency, severity and relationship with treatment of adverse events recorded in the CRD of the participating patients. I know will study using descriptive statistical techniques, such as frequency tables and contingencies.

Tumor Activity: Using RANO criteria, Progression Free Survival, survival rate progression-free at 6 months and response rates in patients with measurable disease.

Global Survival (OS): Median of global survival. Time from start of treatment to trial to date of exitus for any cause. In those patients who are alive in the last follow-up, the OS will be censored at the date of the last follow-up in which the patient was alive. The median OS will be estimated using Kaplan Meier curves.

Changes in the use of corticosteroids: Percentage of patients who have increased / decreased the dose corticosteroids.

Changes in neurological status: Percentage of patients free of neurological impairment in both arms (MMS / Barthel score).

MGMT gene methylation: Effects of MGMT gene methylation on study results. Correlation of laboratory information with clinical information, response to treatment, overall toxicity and survival. This correlation will be studied using statistical techniques descriptive, such as frequency tables and contingency tables.

Statistic analysis
The main objective is to demonstrate that prolonging the treatment until 12 cycles does not improve the progression-free survival at 6 months in the patients included in this study, randomized according to the methylation status of the MGMT and by residual disease or not, to receive 6 additional cycles of temozolomide.

To determine the efficacy of the treatment, progression-free survival, the rate of response and overall survival. To estimate total survival (and progression-free survival) the Kaplan and Meyer nonparametic method. For the univariate comparison of the survival according to the different variables of potential prognostic effect, the test of Mantel-Cox (log rank test). Finally, to carry out a mutivariate survival analysis and have relative risk estimators adjusted for potential confounding variables models will be used of Cox regression.

Based on data from the PIVOTAL study, a 6-month PFS probability of 0.593 is expected in methylated, of 0.285 in non-methylated. We assume 95% confidence, 80% power, and using the comparison formula in the Kaplan-Maier method and log-rank.

First, a descriptive study of the main characteristics of the global series will be carried out. For the continuous variables the mean and standard deviation will be calculated and for the qualitative variables percentages of the corresponding categories will be given.

To carry out the analysis of survival and progression-free survival, we will use the Kaplan-Meier method and, in case of group comparison, the log-rank test with 95% CI. For this type of analysis, the SPSS v15.0 software will be used.
Progression Free Survival (SLP): Time from start of trial treatment to the date of the first progression according to the RANO criteria (Annex IV of this protocol), or death for any reason. Those patients who are alive and have not progressed in the last
follow-up, progression date will be censored to the date of the last follow-up.
Finally to make a mutivariate survival analysis and having relative risk of confusion, estimators adjusted to variables
and Cox regression models will be used.

Total survival (OS) is defined as the time elapsed from randomization to death from any cause. The ST of the living patients at the time of the analysis will be censored in the last follow-up date. The global survival median will be estimated, with their respective 95% confidence intervals. Values of $p < 0.05$ will be considered statistically significant.

Toxicities: The safety and tolerability of the study medication will be determined by evaluating the type, incidence, severity, frequency, severity and relationship with the treatment of events. Adverse events collected in the clinical records of the participating patients. It will be studied using techniques of descriptive statistics, such as frequency tables and contingency tables.

Qualitative variables will be represented by frequency and percentage, continuous variables will be represented such as medians and ranges.

Biomarkers: The frequency of genetic rearrangement will be determined with an interval of 95% confidence. Correlation with survival data will be determined using the method from Kaplan-Meier.

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SPONSOR PROTOCOL NO.: GEINO 14-01

EUDRACT: 2014-000838-39 VERSION: 1.2, dated 8 September 2014

SPONSOR: Spanish Group of Research in Neuro-Oncology - GEINO COORDINATING

INVESTIGATORS:

1. PATIENTS INCLUDED BY CENTER

subject_hospital

	Frequency	Percentage %	Valid %	Cummulative %
Hospital name				
Hospital name				
Hospital name				
Total				

Number patients included:

Number patients that started treatment:

Reasons not to start:

Cause of lack of substudy

		Frequency	Percentage %	Valid %	Cummulative %
	Reason				
	Reason				
	Total				

1.1 METHYLATION STUDY

Assessed locally:

Assessed centrally:

Number of patients:
Methylated:
Unmethylated:
% Methylated:
% Unmethylated:

1.2 RESIDUAL DISEASE DESCRIPTION

Residual disease was considered and enhancement with a diameter largest than 10mm. It was a factor of stratification. There was no central review of images.

	Frequency	%
No Residual disease		
Residual disease		
Total		

1.3 DISTRIBUTION OF PATIENTS ACCORDING TO THE TWO STRATIFICATION FACTORS

ARM	MGMT status	Pts needed	Pts included	Residual Disease	Pts needed	Pts included
TMZ	Met					
	UnMet					
CONTROL	Met					
	UnMet					

1.4 FLOWCHART OF PATIENTS

2. PATIENT CHARACTERISTICS

	[ALL] N=	CONTRO N=	EXPERI N=	p.overall
TREATMENT ARM				
CONTROL				
TMZ				
Sex				
Female				
Male				
Age				
Residual disease>10mm				
No				
Yes				
KPS				
<70%				
>=70%				
Residual Neurological symptom				
No				
Yes				
DXM dose at inclusion				
0mg				
0.5-2mg				
>2				
Barthel index				
0				
1				
MMSE				
<27				
≥27				
NP/ND				

Anticonvulsant therapy				
No				
Yes				
TREATMENT AT DIAGNOSIS PREVIOUS TO ENROLLMENT.				
Initial surgery- TREATMENT AT DIAGNOSIS.				
Biopsy				
Complete resection by post-op MRI)				
Complete resection without post-op MRI				
Subtotal resection				
ADJUVANT TMZ x 6 cycles				
Rxt dose (media)				
MOLECULAR DATA				
Methylation				
Methylated				
Unmethylated				
IDH				
IDH1-R132 MUTATED BY IHC				
IDH1-R132 non MUTATED by IHC				
IDH1-R132 mutated by sequencing				
IDH2-R172 mutated by sequencing				
TOTAL IDH (IHC+SEQ)				
<= 55y old with incomplete IDH data[a]				

2.1 PATIENT STATUS AT THE TIME OF THE REPORT

	Frequency	%	Valid %
No Progression			
Progression			
Total			

	Frequency	%	Valid %
No exitus			
Exitus			
Total			

3. TOXICITY

3.1 BY NUMBER OF CONTROLS/VISITS:

Number of controls/visits

Total:

Control arm:

Treatment arm:

HEMATOLOGICAL TOXICITY

		CONTROL	TMZ	%CONTROL	%TMZ
HEMATOLOGICAL					
Anemia	G1				
	G2				
	G3				
	G4				
Leucocytes	G1				
	G2				
	G3				
	G4				
Neutrophils count	G1				
	G2				
	G3				
	G4				
Platelets	G1				
	G2				
	G3				
	G4				
Lymphocytes	G1				
	G2				
	G3				
	G4				

ADVERSE EVENTS

	CONTROL		TMZ		CONTROL%		TMZ	
	G1 & 2	G3&4	G1&2	G3	G1&2	G3&4	G1&2	G3&4
Type								

LABORATORY INVESTIGATIONS

	CONTROL		TMZ		CONTROL %		TMZ%	
	G1 & 2	G3&4	G1&2	G3	G1&2	G3&4	G1&2	G3&4
Creatinine								
FALCALINA								
K								
GGT								
GOT								
GPT								
BT								
GLUCOSA								
NA								

3.2 BY PATIENT.

	CONTROL N= (%)			TMZ N= (%)		
	Any	G1&2	G3&4	Any	G1&2	G3&4
Leukopenia						
Neutropenia						
Anemia						
Lymphopenia						
Thrombocytopenia						
Creatinine						

Alkaline Phosphatase						
Potasium						
Sodium						
GGT						
GOT						
GPT						
Bilirrubin						
Hyperglycemia						
Neurologic						
Nausea and vomiting						
Asthenia						
Anorexia						
Hearing loss						
Skin						
Anxiety						
Pain						
Constipation						
Respiratory						
Infection						
Thromboemolic						
Bone-events						
Cardiac						
Second-neoplasia						
Hospitalization due to AE						
Patients who needed a TMZ dose reduction						
Patients who needed a delay in cycles						
Patients who withold TMZ due to AE						

3.4 RELEVANT DIFFERENCES IN TOXICITY BY ARM

	CONTROL N= (%)			TMZ N= (%)			P for any
	Any	G1&2	G3&4	Any	G1&2	G3&4	
Lymphopenia							
Thrombocytopenia							
Nausea and vomiting							
Fatigue							
Leukopenia							
Hospitalization due to AE							
Second neoplasia							

The table includes adverse events that will be different between arms

4. SECOND-LINE THERAPIES

	[ALL] N= (%)	CONTROL N= (%)	TMZ N= (%)	p. value overall
Type				
Type				
Type				
Type				
Type				

5. OUTCOME

Median follow-up in months.

5.1 PRIMARY ENDPOINT: DIFFERENCES IN PROGRESSION-FREE SURVIVAL AT 6 MONTHS.

Treatment ARM	6m-PFS	95% CI
Control		
Experimental		

5.2 PROGRESSION FREE SURVIVAL

5.2.1 MEDIAN PFS ALL PATIENTS.

Median PFS			
		95 % CI	
Median	SE	Lower limit	Upper limit

5.2.2.MEDIAN PFS BY TREATMENT ARM

ARM	Median			
	Estimate	SE	95 % CI	
			Lower limit	Upper limit
CONTROL				
EXPER.				
Global				
The estimate is limited to the longest survival time, if censored.				

Figure Kaplan Meier PFS, total and by treatment arm.

	Chi-cuadrado	gl	Sig.
Log Rank (Mantel-Cox)			

5.2.3 MEDIAN PFS BY METHYLATION STATUS

Methylation	Median	95 % CI	
		Lower limit	Upper limit
Met			
Unmet			
Global			

Global comparison

	Chi-cuadrado	gl	Sig.
Log Rank (Mantel-Cox)			

5.2.4 MEDIAN PFS BY RESIDUAL DISEASE

Median and 95% CI for each stratification group.

5.2.5 MEDIAN PFS DEPENDING ON ARM AND METHYLATION STATUS

Median and 95% CI for each stratification group.
Figure of methylated patients according to arm.

Figure of unmethylated patients according to arm

5.3 OVERALL SURVIVAL

Number of deaths.
Total:
By treatment arm:

Median follow up: (months)

		95 % CI	
Estimate	SE	Lower limit	Upper limit

The estimate is limited to the longest survival time, if censored.

5.3.1 OVERALL SURVIVAL BY TREATMENT ARM

Median OS (months).

Total:

By treatment arm:

Figure Kaplan Meier for OS.

5.3.2 OVERALL SURVIVAL DEPENDING ON METHYLATION STATUS.

Patients OS stratified by methylation status.

		95% CI	
MGMT	Estimate	Lower limit	Upper limit
Methylated			
No methylated			
Global			

The estimate is limited to the longest survival time, if censored.

Figure Kaplan Meier OS by stratification groups.

5.3.3 OVERALL SURVIVAL DEPENDING ON RESIDUAL DISEASE

Median OS of patients stratified by residual disease and treatment arm.

Residual disease status		95 % CI	
	Estimate	Lower limit	Upper limit
No			
Yes			
Global			

The estimate is limited to the longest survival time, if censored.

Global comparison

	Chi-cuadrado	gl	Sig.
Log Rank (Mantel-Cox)			

Figure: overall survival for patients depending on residual disease

5.3.4 OVERALL SURVIVAL DEPENDING ON ARM AND METHYLATION STATUS

Median OS of patients stratified by methylation status and treatment arm.

Figure: overall survival of methylated patients depending on arm

Figure: overall survival of un methylated patients depending on arm.

5.4 MULTIVARIATE COS ANALYSIS OF PROGRESSION FREE SURVIVAL AND OVERALL SURVIVAL DEPENDING ON STRATIFICATION FACTORS AND TREATMENT ARM

	Multivariate Analyses					
	Progression-free Survival			**Overall Survival**		
	HR	95%CI	*P*	HR	95%CI	*P*
Experimental arm						
***MGMT* methylation**						
Absence of measurable disease						
***IDH* wild-type**						
***IDH* status not available**						

5.5. HAZARD RATIO WITH FOREST PLOT FOR A PROGRESSION FREE SURIVAL AND B OVERALL SURVIVAL

Forest plot of Hazard ratios of Methylation and residual disease status over PFS and OS.

***MGMT* methylation analysis**

MGMT methylation status will be assessed at the center of origin and centrally.

Immunohistochemistry (IHC) analysis of the mismatch repair (MMR) deficiency proteins (MLH1, MSH2, MSH6, PMS2) and IDH1-R132H

All cases will be reviewed to confirm glioblastoma. Four tissue microarrays (TMA) will be prepared. In samples where hematoxylin-eosin stained whole sections were determined to be non-necrotic, centrally located (non-infiltrating border) areas of the tumor will be selected for TMA construction. Three cylindrical cores, each measuring 0.6 mm in diameter, will be extracted from every donor block using an MTA-1 TMA workstation (Beecher Instruments, Silver Spring, MD) and placed in paraffin receptor blocs.

We will study the protein expression of the mutated form of IDH1-R132H and the expression of the proteins codified by the four genes that regulate the MMR system: mutL homologue 1 (MLH1), mutS homologue 2 (MSH2), mutS homologue 6 (MSH6) and post-meiotic segregation increased 2 (PMS2). For each determination, 4µ-thick sections of the four different TMAs will be obtained, placed on slides with electrostatic charge, and baked for 30 min at 60°C for proper adhesion of the tissue to the slides. Benchmark ULTRA immunostaining automated system (Ventana Medical System Inc., Tucson, AZ) will be used for all IHC determinations. Primary antibodies used will be IDH1-R132H (clone H-09; Master Diagnostic, Granada, Spain) and MLH1, MSH2, MSH6 and PMS2 (clones M1, G219-1129, SP93 and A16-4, respectively; Ventana Medical System Inc, Tucson, AZ).

For IDH1-R132H the IHC result was considered positive if tumor cytoplasmatic positivity was seen.

***IDH* sequencing**

Genomic DNA from tissue will be obtained using the QIAamp® DNA micro kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. *IDH1* and *IDH2* exon 4 will amplified independently with Ecotaq (Ecogen, Barcelona, Spain) using the primers described by Horbinski et al. Cycling PCR conditions:

Sequencing will be carried with ExoProStar™ 1-step (GE Healthcare UK Ltd, Little Chalfont, United Kingdom) and sequenced with the BigDye® Terminator v3.1 Sequencing kit (Applied Biosystems, Foster City, CA) following the manufacturer's instructions with an annealing temperature of 58ºC. The sequencing product will be purified with ZR DNA Sequencing clean-up kit™ (Zymo Research, Irvine, USA) and analyzed in an AbiPrism 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA).

Table: Immunohistochemistry results for mismatch repair proteins in the present study and in previously reported studies

	MLH1 ***N* =**	**PMS2** ***N* =**	**MSH2** ***N* =**	**MSH6** ***N* =**
Not evaluable				
Negative				
<70% positive cells (present study)				
<70% positive cells (Indraccolo)				
<80% positive cells (Felsberg)				
***MGMT* methylation status of patients with ≤80% positive cells (present study)**				

7 .ABSTRACTS AND PUBLICATIONS OF THIS STUDY.

List of communications in Congress (oral and poster).
List of publications in scientific juornals.