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.

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:

Dr Carmen Balañá. Catalan Institute of Oncology (ICO) Germans Trias i Pujol, Badalona

Dr Mª Ángeles Vaz. Ramón y Cajal University Hospital, Madrid.

PROTOCOL SIGNATURE SHEET

Protocol Code: GEINO 14-01

EudraCT No: 2014-000838-39

Version: 1.2, dated 8 September 2014

I have read this protocol and agree to conduct it in accordance with the Good Clinical Practice (GCP) guidelines and the Declaration of Helsinki.

Trial Coordinators	
Dr Carmen Balañá Coordinator's signature	Dr Mª Ángeles Vaz Coordinator's signature
Sponsor Dr Miguel Gil Gil GEINO Chair	

I have read this protocol and agree to conduct it in accordance with the Good Clinical Practice (GCP) guidelines and the Declaration of Helsinki.

Principal Investigator:

Name: .....

1. SYNOPSIS

1.1. Trial

A phase II randomized clinical trial with different durations of adjuvant therapy after the standard first-line treatment.

1.2. Sponsor

SPANISH GROUP OF RESEARCH IN NEURO-ONCOLOGY - GEINO

Registered Office: C/ Velázquez nº7, 3 planta - 28001 Madrid

Contact details:
Secretaría Técnica GEINO
C/ Secretario Coloma 64, esb B, entlo 5ª
08024 Barcelona
Tel.: (+34) 93 434 44 12
Fax: (+34) 93 253 11 68
Email: secretaria@geino.es

1.3. 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.

1.4. Sponsor code

GEINO 14-01

1.5. EudraCT No.

2014-000838-39

1.6. Clinical trial coordinators

Dr Carmen Balañá
Medical Oncology Department,
Catalan Institute of Oncology Badalona
Germans Trias i Pujol, Badalona.
Crta. Canyet s/n 08916, Badalona

Dr Mª Ángeles Vaz
Department of Medical Oncology
Ramón y Cajal University Hospital
Crta. de Colmenar km 9,100
28034 Madrid

1.7. Central study coordinator

Dr Cristina Carrato
Department of Anatomical Pathology
Hospital Germans Trias i Pujol

1.8. Participating sites and Investigators

The Clinical Trial will be conducted with the participation of members from the Spanish Group of Research in Neuro-oncology (GEINO). A comprehensive list of the Investigators, sites and Ethics Committees is available as a separate document.

1.9. Name of the Organisation in charge of monitoring

MARKETING FARMACÉUTICO & INVESTIGACIÓN CLÍNICA – MFAR S.L.
Calle Secretari Coloma 64-68, esc.B, entlo. 5ª
08024 – Barcelona
Tel.: +34 93 434 44 12
Fax: +34 93 253 11 68
Email: investigacion@mfar.net

1.10. Study treatment

Patients diagnosed with glioblastoma will be randomised after receiving 6 cycles of adjuvant therapy with temozolomide (standard treatment) to continue treatment with temozolomide for another 6 cycles or not.

Treatment Groups:

Experimental Arm: Temozolomide 150-200 mg/m^2/d x 5 days every 28 days, for 6 cycles.

Control Arm: No treatment.

Drug presentation: Temozolomide (Temodal®)

. Pharmaceutical form: Hard capsules. Each capsule contains 5 mg, 20 mg and 100 mg of temozolomide.

The hard capsules have an opaque white body, an opaque green cap, and are imprinted with black ink. “Temodal” is printed on the cap.

. *Route of administration*: oral

. *Excipients*: Capsule content: 132.8 mg of anhydrous lactose, colloidal anhydrous silica, sodium starch glycolate, tartaric acid, stearic acid. The capsule shells contain: gelatin, titanium dioxide (E 171), sodium lauryl sulphate, yellow iron oxide (E 172), red iron oxide (E 172) and are imprinted with black pharmaceutical ink, which contains: shellac, propylene glycol, purified water, ammonium hydroxide, 75 potassium hydroxide, and black iron oxide (E 172).

The medicine used in the trial for the experimental arm will be the same as the one used in the first 6 cycles of adjuvant therapy, according to the routine practice of each site, and will not be provided by the trial Sponsor given that continuation of up to 12 cycles constitutes a normal procedure in most of the sites participating in the trial.

1.11. Trial phase and design

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.

1.12. Study objective

The primary objective of the study is to detect differences in the probability of 6-month progression-free survival among patients with methylated or unmethylated MGMT who received 6 additional cycles of temozolomide, stratifying the results by the presence or absence of residual disease.

1.13. Disease under study

Patients diagnosed with glioblastoma will be included.

1.14. Primary endpoint

The primary endpoint of the study to detect differences between the two treatment groups will be 6-month progression-free survival. Said endpoint will be assessed in glioblastoma patients who have already received 6 cycles of temozolomide (adjuvant therapy) without progression, who are randomised to either proceed with 6 additional cycles of temozolomide or to discontinue treatment from the randomisation date until progression, as defined in the RANO criteria.

1.15. Sample size

A total of 160 patients with Glioblastoma will be included. The patients will be stratified by their MGMT methylation status and the presence of residual disease (visible on MRI) upon enrolment.

1.16. Duration of treatment

Treatment in patients randomised to the experimental arm will continue until they have completed 6 additional cycles (a total of 12 cycles of adjuvant therapy) or until disease progression, unacceptable toxicity, non-compliance, withdrawal of consent by the patient or upon the decision of the Investigator, whichever occurs first.

1.17. Estimated study duration

3 years: 2 years of enrolment and 1 year for data analysis.

Expected dates

Expected start date: third quarter of 2014

Expected first enrolment date: fourth quarter of 2014

Recruitment period: 24 months

Expected end date: Second trimester of 2017

2. CONTENTS

3. BACKGROUND INFORMATION

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.

PROTOCOL NO.: GEINO 14-01

EudraCT No.:2014-000838-39

3.1. Type of clinical trial

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.

3.2. Description of the investigational medicinal product

Patients diagnosed with glioblastoma will be randomised after receiving 6 cycles of adjuvant therapy with temozolomide (standard treatment) to continue treatment with temozolomide for another 6 cycles or not.

Treatment Groups:

Experimental Arm: Temozolomide 150-200 mg/m^2/d x 5 days every 28 days, for 6 cycles.

Control Arm: No treatment.

Drug presentation: Temozolomide (Temodal®) Pharmaceutical form: Hard capsules. Capsules containing 5 mg, 20 mg and 100 mg of temozolomide.

The hard capsules have an opaque white body, an opaque green cap, and are imprinted with black ink. “Temodal” is printed on the cap.

3.3. Sponsor details

Spanish Group of Research in Neuro-oncology – GEINO
C/ Velázquez nº7, 3 planta
28001 Madrid

Contact details:
Secretaría Técnica GEINO
Secretari Coloma, 64-68, esc. B, entlo. 5ª
08024 Barcelona
Tel.: + 34 93 434 44 12
Fax: + 34 93 253 11 68
Email: secretaria@geino.es

3.4. Name of the Organisation in charge of monitoring

MARKETING FARMACÉUTICO & INVESTIGACIÓN CLÍNICA – MFAR S.L.
Calle Secretari Coloma 64-68, esc.B, entlo. 5ª
08024 – Barcelona
Tel.: +34 93 434 44 12
Fax: +34 93 253 11 68
Email: investigacion@mfar.net

3.5. General Coordinating Investigator of the clinical trial

Dr Carmen Balañá (ICO Badalona) and Dr Mª Ángeles Vaz (H. Ramón y Cajal) will be the Coordinating Investigators of the trial. They will be responsible for preparing the analyses, results, conclusions and final report.

3.6. Central study coordinator

Dr Cristina Carrato - Dr Carolina Sanz
Department of Anatomical Pathology
H. Germans Trias i Pujol

3.7. Participating sites

A comprehensive list of the participating sites is available as a separate document.

3.8. Estimated study duration

3 years: 2 years of enrolment and 1 year for data analysis.

Expected dates:

Expected start date: third quarter of 2014

Expected first enrolment date: fourth quarter of 2014

Recruitment period: 24 months

Expected end date: first quarter of 2017

4. TRIAL OBJECTIVES

4.1. Background

4.1.1 Epidemiology of glioblastoma

Gliomas are the most common tumours occurring in the central nervous system (CNS), with an annual incidence rate of 5.4 cases per 100,000 inhabitants. According to European statistics and our national records, 54% of these are glioblastomas (GBs). It is estimated that approximately 1,376 patients are diagnosed with the disease every year in Spain. The prognosis is inexorably ruthless, with a median survival of 15-18 months and only 10% of patients alive after 5 years. GB (grade IV astrocytoma; WHO 2007) is histologically characterised by the presentation of nuclear atypia and mitotic activity (traits that define grade II and III astrocytomas, respectively), as well as microvascular proliferation and/or necrosis. It mainly occurs in the cerebral hemispheres. The majority of cases develop *de novo*, with no evidence of a prior precursor lesion, and are known as primary GBs. Secondary GBs develop from lower grade (II or III) astrocytomas. Despite being morphologically similar, these two groups present a different molecular profile and clinical characteristics. Low-grade tumours that become high-grade often present mutations in the IDH1 and IDH2 genes, which encode isocitrate dehydrogenase enzymes. IDH1/IDH2 mutations in *de novo* or primary glioblastoma confers a better prognosis.

Within GB there is a described and validated prognostic index known as the Recursive Partitioning Analysis (RPA). Taking into account the patient's age, initial Mini-Mental State, previous resection and Karnofsky Performance Status, it provides a prognosis according to the benefit of cancer treatment and allows for a comparison of results between studies.

4.2. Glioblastoma treatment

Initial treatment involves surgery to obtain a histological diagnosis and immediately decompress the brain. Surgery must attain the maximum possible resection, whilst also maintaining optimum neurological function to preserve the patient's quality of life. The patient's age and general condition (KPS) are both taken into account when selecting the surgical treatment. Post-operative radiotherapy is the most traditional treatment as it was shown to increase survival from 6 to 8 months in early trials.

The European Organisation for Research and Treatment of Cancer (EORTC) and the National Cancer Institute of Canada (NCIC) performed a randomised phase III trial comparing standard radiotherapy (60 Gy focal radiation over 6 weeks) with the same treatment plus concomitant temozolomide (TMZ) followed by 6 monthly cycles of adjuvant TMZ. 573 patients were included in the study, and the median survival was 14.6 months with the combined treatment versus 12.1 months in the control group. Two-year survival increased from 10.4% to 26.5% in the group treated with TMZ (HR 0.63; $P < 0.001$). The toxicity was tolerable with grades III-IV haematological toxicity in 7% of patients in the concomitant phase and 14% in the adjuvant phase.

The same benefit was proven in a smaller phase II study. After 5 years of follow-up, the results once again confirmed the benefit on survival for the group of patients treated with TMZ and the RPA prognostic value. No other study has improved these results. Concurrent and adjuvant therapy with 6 cycles of TMZ was established as the standard post-surgery treatment in 2005.

TMZ is a second-generation imidazotetrazine derivative that spontaneously undergoes hydrolysis to the active metabolite under physiological conditions and acts as a DNA-methylating agent. It is administered

orally and penetrates the blood-brain barrier (BBB).

Due to their mechanism of action, it is known that cells with the capacity to repair methylated DNA to unmethylated DNA through, among other enzymes, O6-methylguanine DNA methyltransferase (MGMT), may overcome DNA lesions and, as such, prevent apoptosis. The enzyme is encoded by the MGMT gene. MGMT may be methylated in GpG islands and incorrectly transcribed, thereby causing enzyme inactivity. MGMT methylation status is a predictive factor with regard to TMZ response. Methylated patients obtain the greatest treatment benefit, although this is of little value to therapeutic decisions in first-line treatment as there is also a marginal benefit in unmethylated patients.

4.3. Rationale

Despite the fact that there is no scientific evidence that prolonging adjuvant therapy beyond 6 cycles benefits patients, the presence of residual disease in all patients before starting adjuvant treatment together with TMZ's excellent tolerability profile has led to the continuation of the treatment beyond 6 cycles, even up to disease progression in some cases. Treatment is prolonged beyond 6 cycles for 5 basic reasons.

1- The excellent tolerability profile of TMZ, seemingly without cumulative toxicity.

2- Almost all GBs recur after first-line treatment. They may occasionally undergo further surgery or radiotherapy in very specific cases, but there is no recognised or standardised effective second-line treatment. Bevacizumab is a promising drug but lacks the authorisation of the European Medicines Agency (despite having the approval of the FDA) for the treatment of recurrent GB. In Spain, some patients may be treated at selected sites under a compassionate use programme. Other drugs such as nitrosoureas may provide some clinical benefits despite having never demonstrated consistent efficacy results prior to the emergence of TMZ.

3- The concept of adjuvant therapy in GB differs to other diseases as surgery is never radical and there is always some form of residual disease following the intervention.

4- It is difficult to assess the efficacy of the treatment as magnetic resonance imaging (MRI) is not objective or reproducible for the assessment of tumours and residual disease, despite being the recommended imaging technique. When contrasted with gadolinium, MRI scans are able to assess tumour size (T1Gd sequences). Moreover, infiltration and swelling may be examined with T2 and T2/FLAIR sequences, together with other imaging methodologies such as perfusion, diffusion and spectroscopy. However, changes in gadolinium uptake are not indicative of tumour growth, but rather of increases in BBB permeability, which may be secondary to chemoradiotherapy-induced necrosis or to changes in dexamethasone dosages, etc. An event known as pseudo-progression has been described after radiotherapy (with or without TMZ). This consists of a false increase in the lesion undergoing radiation and may occur in up to 30% of patients treated with TMZ and radiotherapy, particularly in the first 3 months following the end of treatment. These false images, which are sometimes accompanied by reversible neurological deterioration, may last for months and confuse viable disease assessment. Pseudo-progression has been associated with greater survival and has also been linked to MGMT methylation. However, it can often lead to a premature and erroneous decision to stop treatment with TMZ, thus impeding any possible therapeutic benefit. Given that it is impossible to differentiate actual residual disease from pseudo-progression, it is recommended that adjuvant TMZ is continued for at least 3 months before any treatment changes. Moreover, if the suspect image persists, treatment is often prolonged beyond the standard 6 cycles.

5- The selection of 6 cycles in the pivotal EORTC/NCIC trial was a random decision that had no bearing on previous experiences.

Choosing 12 cycles of adjuvant TMZ instead of 6 is preferable based on the opinion that 6 cycles may not be enough to control a disease where tumour remnants are still present following adjuvant therapy. In the USA, first-line clinical trials have been designed with the administration of 12 cycles (RTOG 0525 and RTOG 0825), and American and Canadian treatment guidelines recommend prolonging TMZ treatment, particularly in case of residual disease. This is not the case in Europe, where 6 cycles are recommended based on the evidence provided by the pivotal EORTC-NCIC trial. However, treatment is usually continued after 6 cycles in most cases, given the lack of effective rescue therapies.

In principle, prolonged treatment should benefit patients presenting MGMT gene methylation, which is a predictive factor for a positive TMZ treatment outcome.

One Spanish study recently published in *Clinical and Translational Oncology* found that 80.5% of healthcare professionals continued treatment beyond 6 cycles: 44.4% did so only in the event of residual disease, 27.8% always administered 12 cycles and 8.3% continued treatment until disease progression. The economic and demographic study estimated that this practice results in a cost of €1.5 million to the Spanish Health System per year. Those participating in the survey felt that in the majority of cases there

was no evidence in favour of continuing or stopping the treatment and that it would be interesting to address the issue relating to optimal treatment duration in a clinical trial. For this reason, researchers applied for a grant from the Instituto Carlos III Health Research Fund, which was awarded for the purpose of conducting the trial presented herein. Despite the fact the trial was originally envisaged to include randomisation and a placebo, the lack of funding to cover the extra cycles and the difficulty in obtaining a placebo identical to the investigational medicinal product rendered a placebo-controlled trial impossible.

4.4. Objectives

Primary objective:

The primary objective of the study is to detect differences in the probability of 6-month progression-free survival among patients with methylated or unmethylated MGMT who received 6 additional cycles of temozolomide, stratifying the results by the presence or absence of residual disease.

Secondary Objectives:

To detect differences in:

- 6-month progression-free survival for each of the stratification factors after enrolment in the study: Methylation status/residual disease

- Progression-free survival (for all patients and per stratification factor) after enrolment in the study

- Overall survival (for all patients and per stratification factor) after enrolment in the study

- Differences in toxicity between both treatment arms

- Study on temozolomide-resistant markers/enzymes

5. DESIGN AND TYPE OF CLINICAL TRIAL

5.1. Phase of development

Phase IIB, open-label, randomised, multicentre study

5.2. Type of control

Non-placebo control.

5.3. Blinding

No blinding.

5.4. Study design

5.4.1. Patient screening

Patients must sign the Informed Consent Form pertaining to the clinical trial before the screening period begins.

Pre-screening may be performed in Cycle 6 of the adjuvant therapy (standard treatment), but patients will not be randomised until:

the MGMT gene methylation status result has been obtained.

a baseline MRI has been performed on the patient to assess the presence of residual disease.

The different procedures performed as part of routine clinical management (e.g. blood tests, imaging tests, etc.) and carried out prior to the date on which the informed consent form was signed, may be used for screening or as baseline results provided that these have been performed as specified in the protocol.

Once the informed consent form has been signed, each patient will be assigned a screening number. Each site will receive a screening form in the Investigator Site File, which will assign the predetermined screening numbers. This document must always remain at the study site in the custody of the research team. This screening number will identify the patients by the procedures necessary to confirm the

suitability of the former for the study (laboratory analyses, centralised imaging tests, centralised pathology review, etc.) An additional document will be added to the Investigator's Brochure, with detailed information on the identification procedures used for patient screening.

5.4.2. Centralised pathology review

Within the trial, two centralised reviews will be performed at the beginning of the study:

1. For confirmation of the diagnosis of glioblastoma: in all cases, a sample of tumour block or at least 15 histological slides with or without haematoxylin and eosin stains must be sent off for analysis.

2. In order to determine the MGMT gene methylation status: If the site of origin determines the MGMT methylation status locally, the site result will be accepted. If the site of origin does not determine the MGMT methylation status, this will be performed in a centralised manner at the Hospital Germans Trias i Pujol. A tumour sample shipment will therefore be required.

In order to participate in the study, the patient must sign the informed consent form pertaining to the clinical trial.

There is also a subsequent sub-study for the determination of resistance proteins (MSH2, MSH6). In order to participate in this associated sub-study (immunohistochemistry study on resistance proteins), a tissue microarray (TMA) must be produced. As such, a sample of tumour block is required in order to produce said TMA for the subsequent immunohistochemistry study. In order to participate in the associated sub-study, the patient must sign another informed consent form, separate to the one regarding the clinical trial.

All samples will be shipped by courier to the central laboratory. The response to the result of the review and the MGMT test will be obtained within a maximum of 7 days, at which point the patient may be randomised. All cases must be confirmed by the external pathologist conducting said reviews. Moreover, although patients may be registered without their review report, they may subsequently be declared ineligible if their diagnosis is not confirmed.

Any surplus slides will be returned to the site of origin together with a report following the centralised pathology review. The paraffin blocks will be returned after the preparation of the TMA. Likewise, all leftover samples will subsequently be returned after the completion of the project.

No tumour samples may be moved from the hospital of origin until the patient has signed the informed consent form. The centralised review for both the pathological confirmation and the MGMT status determination will be performed by:

Dr Cristina Carrato - Dr Carolina Sanz
Hospital Germans Trias i Pujol
Department of Anatomical Pathology
Crta. Del Canyet s/n - Badalona

To ship the sample, please contact:

MFAR, S.L. - GEINO Technical Secretariat
Secretari Coloma, 64-68, esc. B, entlo. 5ª
08024 Barcelona
Tel.: +34 93 434 44 12
Fax: +34 93 253 11 68
Email: investigacion@mfar.net - secretaria@geino.es

No enrolments will be possible until:

1- The site has the MGMT gene methylation status results, regardless of whether these are the result of a local or centralised tumour sample review (centralised reviews will be performed within approximately 7 days).

2- Prior to signing the ICF (around Cycle 6 of adjuvant therapy), all patient must show an MRI scan showing a lack of radiological progression. The presence of any residual tumour or an image consistent with a residual tumour must be noted on the patient registration sheet for stratification in the trial.

5.4.3. Patient enrolment

Upon receiving confirmation that the patients are eligible for enrolment in the study (MGMT methylation result, MRI showing no progression and compliance with the inclusion/exclusion criteria), the patients will be randomised and assigned a study number in a centralised manner.

The patient enrolment procedure is described below:

1. Fill in and sign the patient enrolment form (registration forms must be signed by a physician (PI or co-PI) identified on the list of signatures and delegation of responsibilities document).

2. Send the completed and signed form to the CRO:

MARKETING FARMACÉUTICO & INVESTIGACIÓN CLÍNICA, S.L.

Fax (+ 34 93 253 11 68) or email to investigacion@mfar.net

3. The CRO will carry out the patient enrolment process.

4. They will respond to the site, sending the patient randomisation confirmation form by both fax and email. The patient randomisation confirmation will contain the patient study number.

5. The treatment arm to which the patient has been randomised will also be disclosed (experimental or control).

6. Treatment may not be commenced without the randomisation result under any circumstances.

The patient study number will identify the subjects throughout their participation in this study.

An additional document will be added to the Investigator's File at the Site, with detailed information on the patient enrolment procedures.

5.4.4. Translational sub-study

In the event the patient has given their informed consent to participate in the translational sub-study, the tumour samples sent for the centralised review and to determine the MGMT methylation status, will be used to prepare a tissue microarray for subsequent immunohistochemistry studies on IDH1 and resistance proteins or TMZ sensitivity (MSH6, MSH2), and any future studies that may arise as a consequence of the trial results.

All samples will be encoded prior to shipment.

An additional document will be added to the Site File, with detailed information on the collection of samples and sample shipment procedures.

6. PATIENT SCREENING

Patients with glioblastoma who meet all the eligibility criteria will be included.

6.1. Inclusion Criteria

Subjects will be considered eligible for enrolment if they meet all of the following criteria:

1. Capacity to understand and sign the informed consent form.
2. Age ≥ 18 years.
3. Patients with glioblastoma according to the WHO classification (glioblastoma) who have received chemoradiotherapy and temozolomide-based chemotherapy (Stupp scheme) and completed 6 cycles of adjuvant temozolomide (with or without bevacizumab) in the context of standard treatment, without disease progression.
4. Availability of tumour tissue from the first surgical intervention for the centralised histology review and in order to determine the MGMT gene methylation status, if this has not been performed at the site of origin (if determined at the site of origin, the site result will be accepted).
5. Stable doses of dexamethasone at enrolment, never exceeding the corticosteroid dose received in Cycle 6 of the adjuvant therapy.
6. Karnofsky Performance Status ≥ 60 %
7. All patients must present an absence of disease progression in a magnetic resonance imaging brain scan, as per the definitions set forth in the RANO criteria prior to randomisation.
8. Baseline study MRI carried out a maximum of 6 weeks prior to enrolment, showing no progression, which may also be administered in treatment Cycle 6. MRIs performed after Cycle 6 of adjuvant therapy are also permissible, provided that no disease progression is observed.

9. Adequate bone marrow reserve: haematocrit ≥ 29%, leucocytes > 3,000/mcl, ANC ≥ 1,500 cells/ul, platelets ≥ 100,000 cells/ul.
10. Creatinine < 1.5 times the upper limit of normal (ULN) for the laboratory performing the analysis.
11. Serum bilirubin < 1.5/ULN; SGOT and SGPT < 2.5 times the upper limit of normal for the laboratory performing the analysis. Serum alkaline phosphatase < 3/ULN.
12. Effective contraceptive method in patients and their partners.

6.2. Exclusion criteria

Subjects who meet any of the following criteria must not be included in the study:

1. Less than 5 years of any previous invasive neoplasia. In situ cervical carcinoma or basal cell skin carcinoma accepted.
2. Concomitant treatment with other investigational agents (except concomitant bevacizumab).
3. Presence of any clinically significant gastrointestinal abnormalities that may affect the intake, transit or absorption of the investigational medicinal product, such as the inability to take tablet-based medicines by mouth.
4. Presence of any psychiatric or cognitive disorder that limits the understanding or signing of the informed consent form and/or impairs compliance with the requirements of this protocol.
5. Concurrent disease that prevents the continuation of temozolomide treatment.
6. Presence of leptomeningeal dissemination.
7. Pregnant or breastfeeding women.
8. HIV-positive patients receiving combined antiretroviral therapy.

6.3. Diagnostic criteria for the study pathologies

Patients who have been diagnosed with glioblastoma and received standard treatment with radiotherapy, temozolomide and a subsequent 6 cycles of adjuvant therapy (with or without bevacizumab) will be selected, provided they have not presented progression, and will be considered for continuing treatment with adjuvant temozolomide for up to 12 cycles.

At that moment, said patients will be randomised to continue or discontinue treatment with temozolomide. To that effect, prior to randomisation, the patients must have an MRI to show the absence of disease progression as well as their MGMT gene methylation result.

6.4. Enrolment

The CRO (MFAR S.L.) must be contacted to proceed with patient enrolment.

Pre-screening may be performed from the beginning of Cycle 6 by means of MRI, provided that the patient is clinically stable. (MRIs performed prior to said cycle will be accepted in order to sign the ICF and begin the enrolment procedures, but this must not exceed a period of 6 weeks).

Patients will not be randomised until:

1. the MGMT methylation status result has been obtained.
2. the baseline MRI scan confirms disease stability.
3. the patient must remain clinically stable without dexamethasone dosage increases. The dose of dexamethasone must be equal to or less than the dose received during Cycle 6 of adjuvant therapy.

Once the patient meets the abovementioned requirements, the patient enrolment sheet will be correctly filled out and sent by fax. MFAR S.L. will then send a fax back to the site confirming enrolment and the assigned treatment arm.

Contact details:

MARKETING FARMACÉUTICO & INVESTIGACIÓN CLÍNICA – MFAR S.L.
C/ Secretari Coloma 64-68, esc.B, entlo. 5ª
08024 – Barcelona
Tel.: +34 93 434 44 12
Fax: +34 93 253 11 68
Email: investigacion@mfar.net

6.5. Expected subjects and calculation of the sample size

A total of 160 patients diagnosed with glioblastoma will be included. The patients will be stratified by their MGMT gene methylation status and the presence of residual disease (visible on MRI) upon enrolment.

6.6. Patient withdrawal criteria

6.6.1. Permanent interruption of the study treatment

The patients will receive the abovementioned treatment until one of the following situations arises:

- Termination of the treatment in accordance with the protocol.
- Termination owing to a decision by the Principal Investigator.
- Unacceptable toxicity or adverse events that could mean, in the eyes of the Investigator, that the administration of the treatment poses an unacceptable risk. In case of unacceptable toxicity, the patients will be monitored until said toxicity has been resolved.
- Disease progression as per RANO criteria.
- Withdrawal of consent by the patient.
- The Investigator or Sponsor feels that the patient no longer meets the study requirements.
- The study ends or is terminated prematurely.
- Major deviations from the protocol:
    - Non-compliance with the inclusion/exclusion criteria.
    - Incapacity to perform comprehensive tumour assessments, as required by the protocol.

The reason behind such treatment discontinuations must be clearly reflected in the case report forms (CRFs).

6.6.2. Study exit

Patients will be encouraged to continue taking part in the study, but may withdraw their consent voluntarily at any time. Moreover, the Investigator may withdraw a patient from the study if he/she sees fit. Lastly, the Sponsor may suspend the trial as and when required.

The reasons behind a patient's early exit from the study must be documented in the CRF as follows:

- The study is closed/complete.
- The patient abandons follow-up.
- Decision by the Investigator.
- The patient withdraws their consent.
- Major protocol breaches.
- Death.

The date and reason for their exit from the study will be listed on the CRF. In the event of death, a death certificate must be obtained (if possible) stating the documented and assessed cause of death. Patients who exit the study may not be reincorporated, regardless of the reason behind their withdrawal.

6.6.3. Procedures to be followed when patients exit the study

The Investigator will adopt the following procedures for all patients exiting the study:

a) In the event of exit due to the end of treatment, the follow-up visits specified herein will be performed.

b) In the event of early withdrawal of the treatment, the patient's subsequent treatment and resulting follow-up will be determined up until death, to ensure they receive medical care in accordance with routine clinical practice.

6.7. Screening failures (screening period)

All patients that sign the informed consent form but withdraw it before their enrolment will be considered screening failures. All potential subjects assessed for enrolment in the study who are deemed screening failures will be recorded in the Identification Listing/Patient Screening Record, but will not be included in the study database. The reasons behind the exclusion of the patients who will not be enrolled in the trial must be recorded on the abovementioned forms. All patients who have undergone randomisation will be registered and documented even if they do not begin prolonged treatment with temozolomide. Said patients will be analysed on an intention-to-treat basis.

7. DESCRIPTION OF TREATMENT

7.1. Treatment regimen

Patients will be randomised in the study (according to their MGMT gene methylation status and the presence or absence of residual disease on the MRI) to one of the following treatment groups:

EXPERIMENTAL GROUP: Temozolomide 150/200 mg/m^2 dose for 5 days every 28 days over 6 cycles (12 cycles of adjuvant temozolomide in total).

CONTROL GROUP: No treatment (6 cycles of adjuvant therapy in total).

The dose that the patient receives in the adjuvant cycles before beginning the trial will be maintained in such a way that the continuation dose may range between 125 mg/m^2/d x 5 days and 200 mg/m^2/d x 5 days.

Treatment must begin no more than 2 weeks after the end of Cycle 6 of adjuvant therapy, i.e. a maximum of 6 weeks after the administration of Day 1, Cycle 6 of the adjuvant therapy is permitted.

Six cycles will be administered, prior to a complete blood count and toxicity test. The dosage level in Cycle 7 (1st additional trial cycle) once the patient has been randomised to the treatment continuation group will be the same as the dose they received previously in Cycle 6 before being enrolled in the trial. We will establish a 0 dose equivalent to the last dose received in Cycle 6.

The patients randomised to the control arm will be visited with the same frequency, as though they had been treated with the 6 additional cycles of temozolomide.

See the treatment regimen in Annex VIII.

7.2. Investigational medicinal product

Temozolomide:

The dose of temozolomide is adapted to the patient's body surface and the total dose may be rounded off to the nearest ten (10 mg).

It will be administered in a single oral dose at least 2 hours before or after any food intake, during the first 5 days of each 28-day cycle. An antiemetic must be prescribed one hour prior to administration on at least 5 days, and the regimen adopted for the initial pre-trial cycles must be followed precisely.

For all phases, any missing doses of the drug due to toxicity adjustments may not be recovered.

7.3. Product bookkeeping and treatment adherence

In accordance with local legislation, the Investigator will indicate in the patient's medical records that he/she has taken the medicine as prescribed for each new cycle initiated in the active treatment arm. The Investigator will also note the dose, whether any doses have been missed and the reason.

7.4. Concomitant medication

All study subjects will be asked to provide a comprehensive list of any drugs they have taken in the 4 weeks prior to the screening period, including both prescription and over-the-counter medicines. The Investigator must be notified of any new drugs administered to the patient from the screening visit up until the post-treatment follow-up visit. Any medicines taken by the patient throughout the course of the study will be recorded in the electronic case report form (eCRF), which will state the indication, dose and administration details regarding the new drug.

Use with caution

Growth factors may be used at the Investigator's discretion to induce increases in the neutrophil count in the event of febrile neutropenia, with a view to administer temozolomide within the scheduled term. Nevertheless, prophylactic use is not permitted.

7.5. Dose modification criteria during the study

7.5.1. General toxicity and dose modifications

Toxicity will be recorded according to the NCIC criteria, version 4.0 (Annex VII). Likewise, adverse effects will be meticulously recorded and reported in accordance with European and Spanish legislation.

7.5.2 Dose adjustment criteria

This phase is considered part of healthcare management but several recommendations are provided below in order to ensure uniform treatment.

The dose that the patient receives in the adjuvant cycles before beginning the trial will be maintained in such a way that the continuation dose may range between 125 mg/m^2/d x 5 days and 200 mg/m^2/d x 5 days. Six cycles will be administered, prior to a complete blood count and toxicity test. The dosage level in Cycle 7, once the patient has been randomised to the treatment continuation group, will be the same as the dose they received previously in Cycle 6 before being enrolled in the trial. We will establish a 0 dose equivalent to the last dose received in Cycle 6.

A reduced dosage level will be permitted for subsequent cycles in case of toxicity, up to a minimum of 125 mg/m^2/d x 5 days.

Cycle 1 (7) dosage level	Dose mg/m^2/d × 5 days	Notes
0	150-200 mg/m^2	Same dose as Cycle 6, prior to enrolment in the trial.
-1	125 mg/m^2	If the patient requires a dose reduction below this figure due to toxicity levels detected in their monthly CBC, they will be forced to abandon the study on the grounds of toxicity.

Dose modification table for subsequent cycles:

Neutrophils 10^9/l	**Platelets 10^9/l**	**Non-haematological toxicity**	**Dose**
≥ 1,500	≥ 100	≤ Grade 2*	Continue treatment at the doses prescribed for that cycle and subsequent cycles
≥ 500 < 1,500	≥25 <100	Grade 3-4	Any of the following: Delay treatment for a maximum of 2 weeks until normal figures are attained. Continue treatment with a one-level dose reduction in the subsequent cycles. If the patient has not recovered after 2 weeks, end adjuvant therapy.
< 500	< 25	GRADE 3-4 not recovered after 2 weeks	Any of the following: Definitive cessation of adjuvant TMZ. Complete blood counts every 2-3 days up until recovery to Grade 3 haematological toxicity (platelets ≥ $25x10^9$/l, neutrophils ≥ $0.5x10^9$/l) and continue with assessment schedule

* except alopecia, nausea and vomiting

8. STUDY DETERMINATIONS AND PROCEDURES

8.1. Determinations during the study

Screening tests must be performed a few days prior to the first administration of the investigational medicinal product for the purpose of enrolling patients in the study.

The following table summarises the procedures and determinations to be performed on both arms throughout the trial as well as the schedule.

All data referring to patients enrolled in this study will be collected in the case report form pertaining to the clinical trial.

1- EXPERIMENTAL ARM

DETERMINATION PATIENTS IN ACTIVE TREATMENT	TREATMENT CYCLE 6 In the previous 28 days	During treatment						After treatment		
		ADJUVANT PHASE cycles/28 d						28 days after end of treatment (end of treatment visit without progression)	Prior to progression (Every 12 weeks)	After progression (Every 12 weeks)
		CYCLE 1 (7)	CYCLE 2 (8)	CYCLE 3 (9)	CYCLE 4 (10)	CYCLE 5 (11)	CYCLE 6 (12)			
Informed Consent	X									
AP Review	X									
MGMT Study	X									
Randomisation	X									
MRI (1)	X			X			X		X	
Medical History	X									
Physical Examination	X	X	X	X	X	X	X	X	X	
CORTICOSTEROIDS (DOSE/DRUG)	X (2)	X	X	X	X	X	X	X	X	
ANTICONVULSANTS (DOSE/DRUG)	X	X	X	X	X	X	X	X	X	
KPS	X	X	X	X	X	X	X	X	X	
TMZ		X	X	X	X	X	X			
Toxicity		X	X	X	X	X	X	X	X	
Clinical Analysis	X	X	X	X	X	X	X	X	X	
Concomitant medication	X	X	X	X	X	X	X	X	X	
BARTHEL INDEX	X			X					X	
MMSE	X			X					X	
CHEST X-RAY (3)	X									
ECG (4)	X									
Patient survival										X

(1) MRI: the baseline MRI will be performed around Cycle 6 (2 weeks before or 3 weeks after) and will be taken as baseline. In case of neurological deterioration, the MRI and response assessment will be brought forward.

(2) Corticosteroids: the baseline corticosteroid dose must not be greater than the dose received during Cycle 6 of adjuvant therapy.

(3/4) The chest X-ray and ECG will only be performed if clinically indicated.

2- CONTROL ARM

DETERMINATION PATIENTS WITHOUT TREATMENT	TREATMENT CYCLE 6 In the previous 28 days	Every 28 days in the 6 months following randomisation	After 6 months post-randomisation	
			Prior to progression (Every 12 weeks)	After progression (Every 12 weeks)
Informed Consent	X			
AP Review	X			
MGMT Study	X			
Randomisation	X			
MRI (1)	X	X	X	
Medical History	X	X		
Physical Examination	X	X	X	
CORTICOSTEROIDS (dose/drug)	X	X	X	
Anticonvulsants (dose/drug)	X	X	X	
KPS	X	X	X	
Toxicity		X	X	
Clinical Analysis	X	X	X	
Concomitant medication	X	X	X	
BARTHEL INDEX (1)	X	X	X	
MMSE (1)	X	X	X	
CHEST X-RAY (2)	X			
ECG (2)	X			
Patient survival				X

(1) The MRI, Barthel Index and MMSE will be performed as per routine clinical practice, every 12 weeks. In case of neurological deterioration, the MRI and Barthel response assessment will be brought forward.

(2) The chest X-ray and ECG will only be performed if clinically indicated.

8.2. Description of procedures

1. Informed consent: each patient must sign the informed consent form prior to undergoing the study-specific assessments and before starting treatment. The patient must sign two separate informed consent forms, one for the clinical trial and another for biological samples.

2. Tumour analysis: After obtaining the patient's informed consent for the tumour sample, tumour tissue from either the original diagnostic biopsy or another recent biopsy will be sent to the central laboratory to confirm the diagnosis. The tumour block should be sent if the MGMT gene methylation status needs to be determined and in order to participate in the study on resistance proteins. In general, paraffin blocks are preferred for the diagnosis confirmation, but 15 unstained slides will also be accepted. The study sample management guide provides details on suitable materials for molecular analysis as well as shipment instructions. These determinations will be performed over approximately 7 days.

* If the site has the MGMT gene methylation status result performed onsite, the centralised review will not be necessary. The methylation result report must be sent along with the tumour sample for the centralised diagnosis review.

3. Inclusion/Exclusion criteria: These must be reviewed in detail for each patient prior to the performance of the study-specific assessments and before starting treatment.

4. Medical history: The patient's general medical history will be examined in the baseline period (in the 2 weeks prior to enrolment), along with a Mini-Mental State Examination (MMSE), a Barthel Index evaluation, Karnofsky Performance Status (KPS) and a neurological deterioration and severity assessment. Details relating to coexisting illnesses will also be collected, along with the patient's cancer history (including smoking habit and history of weight loss in the last 6 months), a description of their first-line treatment with the radiation dose, number of temozolomide cycles and whether or not bevacizumab was administered concomitantly.

5. Full physical examination:

5.1 Pre-treatment (Screening): Physical examination and vital signs (resting pulse, blood pressure, respiratory rate and temperature measurements). The patient's functional status will be assessed using the Karnofsky scale (see Annex I of this protocol). Neurological and functional impairment will be assessed using the MMSE and BI (see Annexes II and III of this protocol). The doses of corticosteroids (dose/drug) and anticonvulsants (dose/drug) will also be recorded

5.2 During treatment and follow-up visits: Physical examination, functional status, KPS and neurological deterioration at each treatment visit (Day 1 of each cycle prior to treatment administration), and at all follow-up visits. Drug, corticosteroid and anticonvulsant dose monitoring.

The BI and MMSE will be measured at the Cycle 1 visit, at each monitoring visit when an MRI is carried out and at the safety visit once the patient has completed the treatment.

6. Concomitant medication: All drugs taken in the four weeks prior to randomisation and concomitantly during the study will be recorded in the patient's medical records and the case report form, stating the indication, dose information and dates of administration. In particular, doses of corticosteroids and anticonvulsants

7. Safety evaluation: Safety and adverse events evaluations comprise signs and symptoms related to the tumour and treatment, as well as those that are unrelated. Adverse events will be documented and recorded in the CRF after being reported by the patients. Specific questions regarding such adverse events will then be formulated. The non-serious AE reporting period shall end 30 days after the last dose of the investigational medicinal product or upon starting a subsequent antineoplastic treatment, whichever occurs first; the serious AE reporting period shall end 30 days after the last dose of the investigational medicinal product, regardless of whether any subsequent antineoplastic treatments have been initiated. Adverse events will be monitored at the follow-up visits occurring after treatment, at least 28 (and no more than 35) days after the end of treatment or up until all drug-related toxic effects have been resolved or are considered irreversible, whichever occurs last. Toxicity will be classified in accordance with the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI CTCAE version 4.0).

8. Clinical analyses: must be performed on Day 1 of each treatment cycle, with a window of up to 72 hours before the scheduled visit.

8.1 Complete blood count: includes a full count of the 3 series: red blood cells, white blood cells (differential count) and platelets.

8.2 Biochemistry: includes glucose, sodium, potassium, creatinine, AST, ALT, alkaline phosphatase, total bilirubin and gamma-glutamyl transferase.

8.3 Pregnancy test (for women of childbearing potential): a blood or urinary pregnancy test (minimum sensitivity of 25 IU/L or equivalent units of beta human chorionic gonadotropin [β-HCG]) in the 7 days prior to enrolment. Subsequently, the blood pregnancy test is only necessary when clinically indicated or if required in accordance with the site's routine clinical practice.

8.4 Coagulation: coagulation tests will be performed if clinically indicated and will include: prothrombin time (PT), partial thromboplastin time (PTT) and fibrinogen.

9. Electrocardiogram (ECG): An ECG will be performed if clinically indicated.

10. Chest X-ray: A chest X-ray will be performed if clinically indicated.

11. Radiological imaging: The baseline and follow-up MRIs will comprise at least T1, T1 Gadolinium and FLAIR sequences on the axial planes, in order to permit compliance with the RANO criteria. It is advisable to use the same apparatus for each patient throughout the study.

12. Treatment adherence: All omitted or missing doses due to vomiting will be indicated in the patient's medical records and CRF, as for the administered doses.

13. Dispensation of medication: Temozolomide will be dispensed on Days 1 to 5 of each 28-day treatment cycle in the active treatment group.

14. Corticosteroid dose: Register the corticosteroid doses the patient received in Cycle 6 of adjuvant therapy in their medical records and the CRF, as well as the baseline dose prior to enrolment. The corticosteroid dose received at each study period must also be recorded in the patient's records and CRF at each visit.

15. Follow-up: All patients will be subject to follow-up in order to determine their subsequent antineoplastic treatments and survival, regardless of the reasons behind the withdrawal of the investigational medicinal product. Information relating to subsequent treatments on progression must include the list of subsequent treatments received by the patient, as well as whether or not the patient has undergone further surgery or radiotherapy. All such information will be noted in the patient's medical records and the CRF.

8.3. Procedures prior to treatment

Prior to carrying out any study-specific procedure, the informed consent form pertaining to the trial must be obtained, except in the case of the MRI, which allows the study to be offered to the patient and is deemed part of treatment, but is also considered as the baseline scan.

Procedures that are a part of regular care are not considered study-specific procedures. Procedures that are a part of regular care can be used as screening procedures to determine eligibility. The eligibility of all subjects should be assessed prior to starting the study treatment. The screening process begins on the date the subject signs the informed consent form approved by the IEC and continues until the randomisation of the patient in the trial and the subsequent administration of the investigational medicinal product. In this study, only eligible subjects will receive the study treatment.

All subjects must have completed the following procedures within 28 days (unless stated otherwise) prior to the first administration of the investigational medicinal product:

- Review of inclusion and exclusion criteria.
- Medical and medication history
- The tumour sample contained in the paraffin block must be identified, prepared and shipped to the central laboratory for molecular analysis of the MGMT gene. The sample may be sent at any time prior to enrolment, even if the patient is still receiving first-line treatment, provided that he/she signs the informed consent form pertaining to the shipment of samples.
- Physical examination, blood pressure, respiratory rate, temperature, weight and height.
- Karnofsky performance status (≤ 14 days prior to enrolment in the study).
- Laboratory analysis and biochemistry tests (≤ 14 days prior to enrolment in the study).
- A blood or urinary pregnancy test for women of child-bearing potential (≤ 72 hours prior to the first administration of the investigational medicinal product).
- ECG and chest X-ray if clinically indicated.
- Barthel Index and MMSE.
- A brain MRI will be taken as a baseline examination for the response assessment (this may be performed within a 6-week period, i.e. an MRI prior to the 6th 15-day cycle, in order to check the clinical stability of the disease). The response must always be assessed using the

same equipment as in the initial examination. In the event the corticosteroid dose has to be increased before the patient starts the treatment, or the patient suffers some form of neurological deterioration during the screening and randomisation period (between Cycle 6 and randomisation), the patient will not undergo randomisation and will be deemed a screening failure. **For that reason, the corticosteroid dose on the day of enrolment may never exceed the dose on the day of randomisation or at the start of treatment.**

Treatment should be initiated as soon as possible in order to allow a time lapse of 4 weeks between Cycles 6 (standard care) and 7 (study), although a delay of up to 2 weeks will also be permitted.

8.4. Tests during treatment

- Experimental Arm

The patients will receive visits every 4 weeks (28-day cycles) over 6 treatment cycles, and will undergo the following at each visit:

- Physical examination: weight
- KPS
- Laboratory analysis and biochemistry tests
- Treatment adherence
- Corticosteroid and anticonvulsant dose
- Toxicity monitoring

If the patient needs to increase their corticosteroid dose or deteriorates neurologically, it is advisable to repeat the initial radiological examination to rule out progression. The corticosteroid dose on the day of enrolment or on signing the ICF may never exceed the dose on the day of randomisation (to rule out progression in said period).

Cycles 3 and 6: the patients will also undergo the following:

- Response assessment regarding the cerebral lesions detected on the MRI, as per the RANO criteria.
- Barthel Index and MMSE.

MRI imaging tests, a Barthel Index test and an MMSE will also be performed every 12 weeks until disease progression; treatment responses (PR, CR) must be confirmed after 4 weeks using the same method and if the patient abandons the treatment for any reason before progression, MRI scans must continue to be carried out every 12 weeks as part of follow-up until progression.

- Control Arm:

The patients will receive visits every 4 weeks in the 6 months following their enrolment in the trial, and will undergo the following at each visit:

- Physical examination: weight
- KPS
- Laboratory analysis and biochemistry tests
- Treatment adherence
- Corticosteroid and anticonvulsant dose
- Toxicity monitoring

If the patient needs to increase their corticosteroid dose or deteriorates neurologically, it is advisable to repeat the initial radiological examination to rule out progression. The corticosteroid dose on the day of enrolment or on signing the ICF may never exceed the dose on the day of randomisation (to rule out progression in said period).

Months 3 and 6: the patients will also undergo the following:

- Response assessment regarding the cerebral lesions detected on the MRI, as per the RANO criteria.
- Barthel Index and MMSE.

MRI imaging tests, a Barthel Index test and an MMSE will also be performed every 12 weeks until disease progression; treatment responses (PR, CR) must be confirmed after 4 weeks using the same method.

8.5. Safety visit

The safety visit will apply to patients treated in the experimental arm, and will be conducted 4 weeks after the end of treatment (regardless of the cause of termination),

- Physical examination and vital signs (resting pulse, blood pressure, respiratory rate, temperature, weight and height).
- Functional status: KPS
- Analysis and biochemistry
- Toxicity monitoring

MRI imaging tests will be performed every 12 weeks until disease progression. Given that the primary objective of the study is progression-free survival, it is essential to rule out progression on the MRIs.

8.6. After end of treatment

- End of treatment without progression:

Once treatment has ended for any reason besides disease progression in the experimental arm or 6 months following enrolment in the control arm, the following procedures will be performed every 12 weeks until disease progression:

- Physical examination and vital signs (resting pulse, blood pressure, respiratory rate and temperature).
- KPS
- Barthel index
- Mini mental state examination.
- Laboratory tests:
- Analysis and biochemistry
- DXM dose and anticonvulsants
- MRI

- After disease progression:

Once the patient progresses, he/she will undergo monitoring for overall survival every 12 weeks up until death. This may comprise visits at the oncology department as well as phone calls. The patient's current status will be determined and any new cancer treatments will be noted (surgery, radiotherapy, chemotherapy and type, and/or bevacizumab, clinical trial).

The date of death will also be verified in order to determine survival.

8.7. Response assessment

RANO criteria will be applied for the response assessment and in order to define progression. Treatment response will be assessed at each site by means of radiology results, the patient's symptoms and the required corticosteroid dose. This will be based on the RANO response criteria.

Progression is defined as:

1. radiological deterioration (increase in the area of contrast uptake or appearance of new lesions).

2. significant deterioration of FLAIR lesions, alongside irreversible neurological deterioration.

3. irreversible neurological deterioration, even in the absence of radiological deterioration.

4. increasing doses of corticosteroids (for more than 2 weeks) to prevent neurological deterioration when dosage reductions are not possible.

8.7.1. Clinical response assessment based on corticosteroids and neurological deterioration

The criteria modified by the RANO Committee are based on radiological response, neurological symptoms and the dose of corticosteroids.

Patients must be kept on the lowest corticosteroid dose they require throughout the treatment (i.e. the dose that keeps them neurologically stable).

In the event a dosage increase is required for any reason (e.g. fever, seizure with a postictal state, etc.), the doses should be returned to the previous dose. In the event a continuous dosage increase is required for more than 2 weeks, the patient shall be deemed to be in clinical progression.

As regards progression with rapid neurological deterioration, a radiological examination is advisable. If this is not possible, brain disease progression will be presumed.

It is important to report the corticosteroid dose received by the patient at the patient assessment visit, as well as the stability or instability of their neurological symptoms and the results of the radiological examination (MRI).

8.7.2 Radiological response assessment

A baseline MRI will be performed in Cycle 3 and every 12 weeks until progression.

The size of the baseline lesion will be assessed at the beginning of the treatment by calculating the product of the 2 largest perpendicular diameters or the sum of the products of the measurable lesions on the axial plane, and will be recorded as the baseline measurement. The measurements taken will be the same as those at baseline. In case of neurological deterioration or the onset of new neurological symptoms, the MRI will be brought forward to determine radiological progression.

The response assessment will analyse contrast uptake and FLAIR hyperintensity separately.

Assessment in T1 Gadolinium sequences

- This will not be the only assessment to define the partial response criteria, stability and progression, as the absence of deterioration in FLAIR sequences should also be taken into account.

- Measurement of the contrast-enhancing component in the axial sections.

- The maximal diameter in the axial section shall be identified, as well as the maximal perpendicular in the same slice.

- Successive tests will employ the same methodology. This will not necessarily correspond to the same slice/diameter performed in the previous study, as lesions often fail to expand symmetrically, but do so by a margin that is not always the same.

- If the lesion comprises different enhancing nodules which are separate but fall within the same hyperintense area in FLAIR, it will be measured as if it were a single nodule.

- This value will be noted in the CRF.

- The appearance of new continuous or scattered enhancing foci shall be noted.

Assessment in T2 FLAIR sequences

Given the difficulty of quantification, a subjective measurement will be performed based on the Radiologist's experience and using the following criteria:

+2.	The lesion presents a clearly superior lesion (at first glance) in comparison to the previous study. As per experiences in other studies, a clear visual difference implies an increase above 20%.
+1.	The lesion presents a larger extension in comparison to the previous study but must be assessed across various slices in order to reach this conclusion.
0.	No changes in the FLAIR extension.
-1.	Reduction in the extension with regards to the previous study
-2.	Clear reduction.

The appearance of new FLAIR hyperintense foci besides those in the initial lesion must be noted. These foci may correspond to new infiltrative areas or to treatment-related changes. The Radiologist's opinion will be indicated on the FLAIR NOTES.

8.7.3. RANO response assessment

Within the scope of this study and given that the treatment response will not be considered a study objective, it is important to determine the patient's **progression date.**

We recommend using the following follow-up table to standardise data collection:

		BASELINE MRI	CONTROL 1	CONTROL 2
DATE				
T1GD	T target	PRODUCT OF DIAMETERS LESIONS 1,2,3...	PRODUCT OF DIAMETERS LESIONS 1,2	ETC
	New foci		YES/NO	YES/NO
Sum of products		MM2	MM2	MM2
FLAIR		VISUAL ASSESSMENT	SAME (0) +1, +2	SAME (0) +1, +2
	NEW FOCI		YES/NO	YES/NO
DXM DOSE		BASELINE DOSE	DOSE	DOSE
SYMPTOMS		BASELINE SYMPTOMS	Stable/Better/Worse	Stable/Better/Worse
RESPONSE			SD/PR/CR/P	SD/PR/CR/P

9. ADVERSE EVENTS

The ICH GCP guidelines require both Investigators and Sponsors to follow a specific procedure when reporting adverse effects or reactions in the context of a clinical trial.

Any event related to drug toxicity, diseases arising during the study or exacerbations of existing conditions must be reported.

Moreover, any clinically significant changes detected in the physical examination or abnormal parameters found as a result of other diagnostic tests (e.g. X-ray, ECG) must also be reported as AEs.

The criteria for classifying a diagnostic test result as abnormal are as follows, and must therefore be classified as AEs:

- the diagnostic test result is associated with clinically significant symptoms, and/or
- the diagnostic test result leads to a change in the dose of the investigational medicinal product or withdrawal from the study, or implies the incorporation of another drug or type of therapy to control the patient's symptoms, and/or
- the diagnostic test result leads to an outcome listed under the SAE definition, and/or
- the Investigator considers the diagnostic test result to be an AE.

9.1. Definitions

The ICH GCP definitions apply to this protocol.

Adverse event (AE)

Any incident that is harmful to the health of a clinical trial patient or subject treated with a medicinal product, even if it does not necessarily have a causal relationship with said treatment.

Adverse Reaction (AR)

An AR is any harmful, unintended reaction to an investigational medicinal product, irrespective of the administered dose.

Serious Adverse Event (SAE) and Serious Adverse Reaction (SAR):

Any adverse event or reaction that, at any dose:

- causes death (is fatal);
- is life-threatening to the patient;
- requires or prolongs hospitalisation;
- causes permanent or significant disability;
- is a congenital anomaly or birth defect, or;
- is clinically significant.

Clinical and scientific judgment must be exercised when deciding whether or not it is appropriate to send an urgent report regarding clinically significant events that are neither life threatening nor require

hospitalisation, but which may put the patient at risk or require an intervention in order to prevent one of the abovementioned outcomes.

All suspected transmissions of an infectious agent through a medicinal product will also be reported as serious.

Suspected Unexpected Serious Adverse Reaction (SUSAR):

A serious adverse reaction that is "unexpected", e.g.: A serious adverse reaction, whose nature and severity is not consistent with the information provided about the pharmaceutical product in question in the Summary of Product Characteristics (or Investigator's Brochure).

The reference document used to establish the "expectability" of the adverse events for temozolomide will be the latest version of the Summary of Product Characteristics available on the EMA website.

Life-threatening event

Any event in which the patient is at immediate risk of death at the time of the event; this does not refer to an event which hypothetically might have caused death if it had been more severe.

Hospitalisation/prolongation of hospitalisation

Any event that requires (or prolongs) hospitalisation and arises or worsens during the patient's participation in a clinical study shall be reported as an SAE. Prolongation of hospitalisation is defined as any extension of the patient's hospital stay beyond the duration envisaged/prescribed on admission, as determined by the Investigator or the doctor responsible for said patient's care.

The following cases of hospitalisation do not meet the SAE reporting criteria:

a) Reasons described in the protocol (e.g. administration of the investigational medicinal product, additional tests required by the protocol). Hospitalisations or prolongations due to complications in administering the treatment or procedures will be reported as SAEs.

b) Hospitalisations or prolongations for technical, practical or social reasons, in the absence of an AE.

c) Previously scheduled hospitalisations (i.e. those arranged before the patient's enrolment in the study). Any surgical interventions or procedures arranged before the patient's enrolment in the study must be documented in the CRF.

Unexpected Adverse Reaction (UAR):

Any adverse reaction whose nature, intensity or consequences do not match the reference information for the medicinal product (e.g. the Investigator's Brochure in the case of an investigational medicinal product that is yet to be authorised for marketing, or the Summary of Product Characteristics in the case of an authorised product).

The reference document used to establish the "expectability" of the adverse events for temozolomide will be the latest version of the Summary of Product Characteristics available on the EMA website.

Associated with the use of the medicinal product

An AE is considered to be associated with the use of the investigational medicinal product if the causality assessment involves any of the investigational medicinal products or is unknown, in accordance with the definitions below.

Causality assessment.

The Investigator shall conduct a causality assessment on each investigational medicinal product (including combination products and comparators) in accordance with the following criteria:

Y There is a reasonable possibility that the investigational medicinal product(s) caused the serious adverse event.

N There is no reasonable possibility that the investigational medicinal product(s) caused the serious adverse event, and other causes are more likely.

UK The cause is unknown. This should only be used in extraordinary circumstances where the Investigator has insufficient information (e.g. the patient was not treated at his/her site), and if none of the previous options are applicable.

9.2 Reporting and documenting Adverse Events

The Sponsor shall gather information on AEs from the date on which the Informed Consent Forms are signed up until 30 days after the administration of the final dose of the investigational medicinal product.

All AEs must be recorded in the source document and the CRF using medical terminology. The Investigators shall assess the severity (grade) of the event in compliance with the NCI-CTC V 4.0, establish the relationship with each of the investigational medicinal products and seek out and obtain suitable information to determine the outcome and to assess whether or not the event meets the

criteria for classification as a SAE, in which case it will be reported immediately. The Investigator shall provide any information requested by the Sponsor, as well as the details recorded on the CRF.

All SAEs (as defined above) arising during the clinical trial or within 30 days of the final dose of the investigational medicinal product shall be reported by the Investigator, irrespective of the suspected relationship with the study treatment. Moreover, any SAEs arising as a result of the diagnostic procedures or interventions specified herein shall also be reported. Beyond this period, only those SAEs suspected to have a causal relationship with the investigational medicinal product shall be reported.

All AEs suspected to be related to the investigational medicinal product shall be monitored after the cessation of the treatment up until the event or its sequelae have been resolved or stabilised to an acceptable level in the eyes of the Principal Investigator, Chief Investigator and/or the Sponsor.

The research team shall report all pregnancies occurring during the clinical trial to the Sponsor, regardless of whether they relate to patients or the female partners of patients, within 24 hours of receiving notice of the same. The outcome of the pregnancy must also be reported within 24 hours of receiving notice of the same.

The cause of death of patients who die during a clinical trial, regardless of whether or not said cause is an expected event or associated with the investigational medicinal product, will be considered an SAE and must therefore be reported using the SAE document. If available, the autopsy report must be exclusively identified with the patient's enrolment number and sent to the Sponsor.

All serious adverse events must be reported by fax within 24 hours to
MFAR, S.L.
Fax: +34 93 253 11 68
Email: investigacion@mfar.net

The SAE report must contain a comprehensive written summary detailing the important aspects of the corresponding adverse events. Where applicable, this should include information regarding the relevant medical records and autopsy reports. Follow-up information must be sent to MFAR, S.L. within the next 24 hours.

All AEs suspected to be related to the investigational medicinal product shall be monitored after the cessation of the treatment up until the event or its sequelae have been resolved or stabilised to an acceptable level in the eyes of the Principal Investigator, Chief Investigator and/or the Sponsor.

9.3. SUSAR reporting by the Sponsor

The Sponsor shall be responsible for reporting SUSARs to the regulatory authorities in an appropriate manner. The procedure set forth in the most recent version of the "Detailed guidance on the collection, verification and presentation of adverse reaction reports arising from clinical trials on medicinal products for human use" document will be adopted, which is available on the EMA website.

All suspected unexpected serious adverse reactions (SUSARs) will be reported in accordance with the current European regulations on clinical trials, to the Competent Authorities, IECs and Investigators, in the terms and manner set forth in "Detailed guidance on the collection, verification and presentation of adverse reaction reports arising from clinical trials on medicinal products for human use" and pursuant to any local regulations that may be applicable.

9.3.1 Terms for reporting SUSARs to the regulatory authorities

The Sponsor shall report any individual SUSAR cases within a maximum period of 15 calendar days from the date on which it learns of said suspected adverse reaction. When the SUSAR has caused the subject's death or endangered his/her life, the Sponsor shall report this information within a maximum period of 7 calendar days from the date on which it learns of the event. Said information shall be completed, as far as possible, within the following eight calendar days. This information shall include an assessment of the significance and implication of these findings, including any previous relevant experiences regarding the investigational medicinal product or similar medicines.

9.3.2 Reporting other important safety information

The Sponsor must report (as soon as possible and no later than 15 days) any information that could modify the risk/benefit assessment regarding the investigational medicinal product, or define changes to be made to the administration regimen or conduct of the trial, e.g.:

- A qualitative change or an increase in the percentage of occurrence of expected serious adverse reactions, which is considered clinically significant.

- SUSARs occurring after the completion of the clinical trial and which should be reported by the Investigator to the Sponsor.

New events related to the conduct of the trial or the development of the investigational medicinal product, which will probably affect patient safety, such as:

- Adverse events that may be related to trial procedures and which could modify the conduct of the trial.
- A significant risk to the subjects, such as the inefficacy of an investigational medicinal product used to treat a life-threatening disease.
- Significant new safety findings from new animal studies (such as carcinogenicity).
- Any early termination or temporary cessation of another clinical trial on the same investigational medicinal product due to safety reasons, carried out in another country by the same Sponsor.
- Clinically significant serious adverse reactions that are exclusively related to non-investigational medicinal products, as these are not subject to the general reporting rules for individual SUSAR cases.

Moreover, in the event further relevant information is obtained, this will be reported as quickly as possible.

9.3.3 Reporting to the Investigators

The Sponsor shall report any information to the Investigators that may affect the safety of the subjects participating in the trial as soon as possible.

Moreover, the Sponsor shall inform the Investigators of any safety aspects that may have an impact on the conduct of the clinical trial or on the development of the product, including interruptions to the development programme or safety-related protocol amendments.

Reporting procedures:

1. The Investigator (or medical consultant in charge of the patient, who is named on the list of signatures and the delegation of responsibilities document) must fill in the SAE form, specifying the grade, causality and "expectability" of the event, as indicated above. In the absence of the Principal Investigator, a member of the site's trial team shall fill in and sign the form. The Principal Investigator must then check the SAE form, implement any appropriate changes, sign it and send it by fax to MFAR, S.L. (tel.: +34 93 434 44 12 – Fax: +34 93 253 11 68 – Email: investigacion@mfar.net) as soon as possible. This initial report will be followed by a detailed written report, where appropriate.

2. Follow-up: The patients must be monitored until their clinical recovery is complete and the laboratory results have returned to normal or baseline values, or until the event has been stabilised. Follow-up must continue until the end of treatment if necessary. The follow-up information must be recorded in an additional SAE form, ticking the follow-up box, and will be sent by fax to the Secretariat when it becomes available. Additional written information and/or copies of test results may also be provided separately. Patients will only be identifiable by their trial number, date of birth and initials. Names must not be used in any communications.

3. MFAR S.L. shall notify the local and regional Independent Ethics Committees (IEC) of the event (in accordance with the standard local clinical trial procedures), as well as the Spanish Agency of Medicines and Medical Devices (AEMPS).

10. STATISTICAL CONSIDERATIONS

10.1. Study variables

10.1.1. Primary efficacy endpoint

The primary endpoint of the study to detect differences between the two treatment groups will be 6-month progression-free survival. Said endpoint will be assessed in glioblastoma patients who have already received 6 cycles of temozolomide (adjuvant therapy) without progression, who are randomised to either proceed with 6 additional cycles of temozolomide or to discontinue treatment from the randomisation date until progression, as defined in the RANO criteria. Those patients who are alive and show no signs of progression at this stage will be considered successful, while those who have progressed or died will be deemed treatment failures. The diagnosis of progression must be based on the RANO criteria.

Disease progression is defined as:

1. radiological deterioration (increase in the area of contrast uptake or appearance of new lesions), i.e. RANO progression criteria.
2. significant deterioration of FLAIR lesions, alongside irreversible neurological deterioration.

3. irreversible neurological deterioration, even in the absence of radiological deterioration.
4. increasing doses of corticosteroids (for more than 2 weeks) to prevent neurological deterioration.

10.1.2. Secondary endpoints

The following aspects will be determined and compared in both treatment arms:

Clinical, biological and demographic data: (sex, age, type of surgery, initial MMSE, Barthel Index, presence of neurological symptoms (mild, moderate, significant), whether bevacizumab was administered in first-line therapy or not, second-line treatments.

Safety/toxicity profile: Type, incidence, severity, frequency, gravity and relationship with the treatment of adverse events recorded in the participating patients' CRF. These aspects will be studied by means of descriptive statistics techniques, such as frequency and contingency tables.

Tumour Activity: As per RANO criteria, progression-free survival, the rate of 6-month progression-free survival and response rates in patients with measurable disease.

Overall Survival: Median overall survival. Time from the start of the trial treatment until the date of death due to any cause. As for those patients who are alive at the final follow-up visit, their OS will be censored on the date of said final follow-up visit in which the patients were alive. The median OS will be estimated using Kaplan-Meier curves.

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

Changes in neurological state: Percentage of patients free from neurological deterioration in both arms (MMSE/Barthel score).

MGMT gene methylation: Effects of MGMT gene methylation on the study results. Correlation between the laboratory information and clinical data, treatment response, toxicity and overall survival. This correlation will be studied by means of descriptive statistics techniques, such as frequency and contingency tables.

10.2. Efficacy evaluation

All randomised patients will be included in the main response analysis: 6-month progression-free survival will be assessed in accordance with the RANO criteria.

Any patients who discontinue the treatment for any reason besides disease progression (toxicity or desire of the patient) must undergo an MRI scan every 12 weeks until disease progression, as part of their routine care. Imaging tests must be performed using the same method on each occasion (magnetic resonance).

Progression-free survival (PFS): Time from the start of the trial treatment until the date of the first progression, in accordance with the RANO criteria (Annex IV of this protocol), or death due to any cause. As for those patients who are alive and have not progressed by the final follow-up visit, their date of progression will be censored on the date of said final follow-up visit.

Disease progression is defined as:

1. Radiological deterioration (increase in the area of contrast uptake or appearance of new lesions), i.e. RANO progression criteria.
2. Significant deterioration of FLAIR lesions, alongside irreversible neurological deterioration.
3. Irreversible neurological deterioration, even in the absence of radiological deterioration.
4. Increasing doses of dexamethasone (for more than 2 weeks) to prevent neurological deterioration.

6-month progression-free survival (PFS) as the percentage of patients who present/do not present disease progression 6 months after starting treatment with the investigational medicinal product.

10.3. Safety evaluation

Any patient enrolled in the trial who has been randomised (having ruled out previous progression) or received at least one dose of the investigational medicinal product will be evaluable for the toxicity analysis.

The safety and tolerability of the investigational medicinal product will be determined by assessing the type, incidence, severity, frequency, gravity and relationship with the treatment of the adverse events reported, physical examinations and laboratory tests. Toxicity will be classified and tabulated using the NCI-CTCAE v 4.0.

Any sign or symptom related to the tumour existing at baseline, which deteriorates (in terms of severity or frequency) during the trial, shall be recorded as an adverse event.

All adverse events must be recorded at each study visit in accordance with NCI-CTCAE version 4.0.

10.4. Study populations

Safety population: The patients enrolled in the study will be those who have received at least one dose of the investigational medicinal product.

Intention-to-treat population (efficacy analysis population): Patient group comprising all subjects who have been randomised in the trial.

Per-protocol population: The patients enrolled in the study will be those who have received one dose of the investigational medicinal product, comply with the inclusion/exclusion criteria, have not progressed upon randomisation or incurred significant protocol deviations during the study.

10.5. Sample size and statistical analysis

10.5.1. Sample size

The sample size is calculated based on the hypothesis defined in the primary objective of non-superiority as regards 6-month progression-free survival in continuation from 6 to 12 months of treatment between methylated and unmethylated patients.

Based on the EORTC-NCIC study data (Stupp et al., 2005, 2009 ref. 3), a PFS probability of 0.593 is expected in the methylated patients, and 0.285 in the unmethylated patients. A 95% confidence interval and 80% statistical power are assumed, and are calculated on a 1:1.5 reasoning due to the fact that there are more methylated patients. With a maximum of 10% losses expected, the sample must comprise 32 unmethylated patients and 48 methylated patients (total n=80) in the TMZ treatment group. In order to respond to the endpoint comparing the 6- and 12-month values, the same sample is established for the group of patients who will not undergo additional treatment with 6 cycles of temozolomide. This leads us to a sample of 64 unmethylated and 96 methylated patients for a total of 160 enrolled patients. (n=160).

Bearing in mind that the patients who present residual disease may draw benefits in comparison to those who do not, this variable is established as a randomisation factor. Moreover, taking into account that 60% residual disease is expected in methylated patients and 40% in unmethylated patients, 4 randomisation groups are established to ensure that 13 of the 32 unmethylated patients and 23 of the 48 methylated patients present residual disease.

10.5.2. Statistical analysis

The primary objective is to show that prolonging treatment to 12 cycles does not improve 6-month progression-free survival in the patients enrolled in the study, who have been duly randomised according to their MGMT methylation status and whether or not they present residual disease, after receiving 6 additional cycles of temozolomide. As such, the primary date is from progression that presents in the form of progression.

In order to determine the efficacy of the treatment, the study will assess progression-free survival, response rates and overall survival.

In order to estimate overall survival (and progression-free survival), the non-parametric Kaplan-Meier method will be used. The Mantel-Cox test (log-rank) will be used for a univariate comparison of the survival curves according to the different prognostic effect variables. Finally, in order to analyse multivariate survival and have relative-risk estimators adapted to confounding variables, Cox regression models will be used.

Based on the PIVOTAL study data, a 6-month PFS probability of 0.593 is expected in the methylated patients, and 0.285 in the unmethylated patients. A 95% confidence interval and 80% power is assumed, and the Kaplan-Meier comparison formula and log-rank test will be employed.

A descriptive study will initially be performed on the main characteristics of the entire patient group. As regards the continuous variables, the mean and typical deviation will be calculated. With qualitative variables, the corresponding percentages will be given, without taking into account any missing values. In order to perform the survival and progression-free survival analysis, the Kaplan-Meier method will be used. When it comes to comparing the groups, a log-rank test will be applied, with a CI of 95%. For this type of analysis, we will use SPSS software (v. 15.0).

Progression-free survival (PFS): Time from the start of the trial treatment until the date of the first progression, in accordance with the RANO criteria (Annex IV of this protocol), or death due to any cause. As for those patients who are alive and have not progressed by the final follow-up visit, their date of progression will be censored on the date of said final follow-up visit.
Disease progression is defined as:

1. Radiological deterioration (increase in the area of contrast uptake or appearance of new lesions), i.e. RANO progression criteria.
2. Significant deterioration of FLAIR lesions, alongside irreversible neurological deterioration.
3. Irreversible neurological deterioration, even in the absence of radiological deterioration.
4. Increasing doses of dexamethasone (for more than 2 weeks) to prevent neurological deterioration.

Progression-free survival will be determined using the non-parametric Kaplan-Meier method. The Mantel-Cox test (log-rank) will be used for a univariate comparison of the survival curves according to the different prognostic effect variables. Finally, in order to analyse multivariate survival and have relative-risk estimators adapted to confounding variables, Cox regression models will be used.

Overall survival (OS) is defined as the time elapsed since randomisation until death due to any cause. The OS of patients who are alive at the time of analysis will be censored at the final follow-up visit. Their median OS will be estimated, using the respective confidence intervals of 95%. P-values below 0.05 will be considered statistically significant.

Toxicities: The safety and tolerability of the investigational medicinal product will be determined by assessing the type, incidence, severity, frequency, gravity and relationship with the treatment of the adverse events recorded in the participating patients' medical records. These aspects will be studied by means of descriptive statistics techniques, such as frequency and contingency tables.

The variables will be represented by frequency and percentage, while continuous variables are represented as medians and ranges.

Biomarkers: We will determine the frequency of genetic reassortment, using a confidence interval of 95%. The correlation with survival data will be calculated using the Kaplan-Meier method.

11. ETHICAL AND LEGAL CONSIDERATIONS

11.1. Ethics Committee

The study will be conducted in compliance with the ethical principles of the Declaration of Helsinki, adopted by the 18th World Medical Association General Assembly, Helsinki, Finland.

In accordance with European Parliament directives 95/46/EC and 2001/20/EC, which regulate the requirements for conducting clinical trials, any information collected throughout the duration of the trial may only be used by the trial Sponsor to assess the results in compliance with the abovementioned directive.

In Spain, the following also apply:

The Oviedo Convention, of 4 April 1997, on human rights and biomedicine, which was ratified in the Official State Bulletin (BOE) in October 1999.

Regulations for adequate personal data protection, in accordance with the provisions of Organic Law 15/1999 on Personal Data Protection.

Rights and obligations with regard to clinical information and documentation, in accordance with Law 41/2002, of 14 November, which sets out the legal provisions for patient autonomy.

The 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996 and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000.

Law 14/2007, of 3 July, on Biomedical Research.

11.2. Authorities

The study protocol and/or the various documents relating thereto must be submitted to the national authorities before the start of the trial, in accordance with the procedures established by the same.

11.3. Informed Consent

The patient shall sign two Informed Consent Forms:

For the clinical trial and the use of their biological samples for the centralised pathology review.

For patient participation in the associated translational sub-study.

The study doctor must explain the nature, objectives and potential consequences of the clinical trial, in a way that is understandable to the patient.

The patient must grant his/her consent before being enrolled in the clinical study and prior to providing biological samples.

The study subject shall grant his/her consent by signing the corresponding template in duplicate. To that effect, each template must bear the signature of both the Investigator and the patient. The Investigator will file and keep a copy of each original Informed Consent Form signed by the patient.

The Investigator will not begin any trial-related research until the patient's consent has been obtained.

The Informed Consent Form used in this study, and any changes made throughout the course of the same, must be subsequently approved by the Ethics Committee.

11.4. Confidentiality

In order to guarantee the confidentiality of the trial data in accordance with the provisions of European Parliament Directive 2001/20/EC, only the trial Sponsor and its representative, for monitoring/auditing tasks, as well as the Investigator, co-investigators, Independent Ethics Committee of the corresponding site, the person overseeing the trial and the pertinent health authorities shall have access to the data.

In the abovementioned case of Monitoring/Audits, the Investigator shall provide direct access to the source documents and data.

The contents of the case report forms (CRFs) and the documents generated during the study will be protected from unauthorised use by non-research related persons and classified as strictly confidential, without being revealed to any parties besides those specified in the previous paragraph.

In Spain, this trial will also be conducted in accordance with the provisions of Organic Law 15/1999, on Personal Data Protection.

11.5. Insurance

An insurance policy will be taken out in accordance with the regulatory requirements of each country where the trial is being conducted.

In Spain, all patients enrolled in the study will be insured by HDI Hannover Internacional (España) Seguros y Reaseguros, S.A., with a policy that fulfils the conditions stipulated in Royal Decree 223/2004.

11.6. Completion of the trial

The trial will be considered complete from a regulatory perspective after the data relating to the primary and secondary endpoints have been sufficiently prepared for the initial publication.

11.7. Premature termination of the trial

This study may be terminated prematurely if there is a reasonable cause, in the opinion of the Sponsor. The Investigator shall receive written notice in which the terminating party shall document their reason for suspending the study. Circumstances that warrant a suspension of the study include, but are not limited to:

- Determination of unexpected risks which are deemed considerable or unacceptable to patients
- Inability to enrol an acceptable number of patients
- Insufficient compliance with the protocol requirements
- Plans to modify, suspend or discontinue the development of the investigational medicinal product
- In the event of premature termination of the trial, all materials (complete, partially complete or blank CRF, investigational medicinal product, etc.) shall be returned to GEINO.

12. PRACTICAL CONSIDERATIONS

12.1. Diagnostic criteria for the disease under study

The patients enrolled in this trial must have received histological confirmation of their glioblastoma diagnosis, as well as their MGMT gene methylation result.

Paraffin blocks/tumour slides will be collected from all patients in order to proceed with the centralised pathology review.

12.2. Investigator's responsibilities as per Good Clinical Practices

The responsibilities of the Principal Investigator at each participating site will be:

1. To sign the trial project.

2. To be fully conversant with the properties of the medicinal products.

3. To obtain the informed consent of the subjects before their enrolment in the trial.

4. To ensure that the patients receive suitable medical care in the event of trial-related adverse events, including significant laboratory test values.

5. To collect, record and report the data correctly.

6. To immediately report serious or unexpected adverse events to the Sponsor.

7. To guarantee that all persons involved respect the confidentiality of all information relating to the trial subjects.

8. To regularly report to the Independent Ethics Committee on the progress of the trial.

9. To be responsible for writing the final trial report, giving their agreement thereto with their signature.

12.3. Instructions for filling in the electronic CRF

The trial data will be recorded in compliance with GCP, by means of an electronic documentation system at the site.

This application is designed to operate entirely via the web. All of the different processing stages – except data entry and visualisation – will be performed centrally via a web server/database. In particular, the data will only be stored in a centralised manner.

For the purposes of entering data and printing results, the system is based solely around a so-called "network interface", i.e. the data entry forms and reports are viewed from the client's computer as HTML pages (Hyper Text Markup Language - the standard description language for internet pages) via a web browser. The user does not need to install any specific software in order to use the system from the Investigator's computer. It is also possible to access the unprocessed data directly via a database on the ODBC application in order to proceed with additional data processing.

The system verifies data correction by ranges, while also performing validity and consistency checks. Implausible or omitted data may be corrected or completed after being reported to the Investigator. Such correction documents shall be stored and kept on record for auditing purposes.

The system has a network of codes and passwords that restrict access to the different areas of the application, depending on the role assigned by the Sponsor. Besides the Investigator, only expressly authorised persons who have received study-specific training may fill in the eCRFs.

All of the data recorded in the eCRFs must be possible to document on measurement logs or by means of annotations on the patients' medical records.

12.4. Final manuscript and publications

Publication of the clinical trial

The Principal Investigators shall appear as the authors of the study, along with other investigators who have provided at least 3% of the patients.

The order of the authors shall be based strictly on the number of patients each Investigator enrols. The Trial Coordinators (Dr Balaña and Dr Mª Vaz) will occupy first place, last place or "corresponding author".

The study will be published by those in charge of the clinical trial, as well as the clinical investigators.

The main author, Principal Investigators and study designer will be responsible for drafting the final publications.

The subjects providing the study samples shall remain anonymous at all times. The results or conclusions of the study shall be published first and foremost in scientific journals, before being disclosed to the general public. Procedures of unconfirmed efficacy shall not be disclosed prematurely or in a sensationalist manner.

The participating investigators must not publish any patient data that is directly related to the study's endpoints until the trial report has been published.

The trial will be registered on the publicly available database www.clinicaltrials.gov

Publication of the translational study

The results of the translational study will be published in scientific journals after the publication of the main clinical trial results.

The main author shall be the Translational Study Coordinator. All sites that have contributed to at least 5% of the analysed material shall be entitled to one co-author upon publication; those sites that have contributed to at least 15% of the analysed material shall be entitled to two co-authors. The co-author(s) shall be selected at the discretion of each site (pathologists, molecular biologists, doctors, etc.). All sites that have provided materials for analysis will be considered in the acknowledgments.

The clinical trial shall be registered at “clinicaltrials.gov”, and in the National Institutes of Health of the United States database.

12.5. Monitoring

The study will be monitored by means of onsite visits, telephone calls and periodic CRF inspections, as often as required to verify the following:

- Patient recruitment rate.

- Compliance with the approved protocol and amendments, if applicable.

- That the Investigator has received the necessary trial documents and provisions in order to carry out the trial in an appropriate manner and to comply with the applicable legal provisions.

- That the Investigator and the Investigator’s team are adequately informed about the trial.

- Integrity and accuracy of the data entered in the CRF (in accordance with the monitoring plan).

- Informed consent (version, date and signature).

- Eligibility criteria.

- Screening tests.

- SAEs and SAE reporting.

- Primary trial endpoint.

- Collection and storage of biological samples.

- Filling in CRFs and recording adverse events.

- Reporting protocol deviations in accordance with Good Clinical Practices and regulatory requirements, taking any necessary actions to prevent the recurrence of the deviations detected.

The monitoring visits will be performed by the study monitors. It is understood that these monitors may access the patients’ medical records after submitting a request to the Investigator. The Investigator shall set aside enough time for these visits and shall provide access to all documentation to the corresponding authorised persons.

12.6. Protocol amendments

Changes or addenda to the protocol may only be made by the Sponsor, who must submit these to the Independent Ethics Committee and Regulatory Authorities (in Spain, the AEMPS) as protocol amendments.

12.7. Data processing

All data (personal, clinical, economic and those resulting from biological material assessments) obtained from the patients shall be treated in accordance with European Parliament Directive 95/46/EC, of 24 October 1995, on the protection of persons with regard to personal data processing.

As established by the aforementioned legislation, the patients may exercise their rights to access, modify, oppose and cancel the data, in which case they must approach the study doctor.

The content of the CRFs, as well as the documents generated during the study, shall be considered strictly confidential and will not be disclosed to third parties.

In Spain, this study will also be performed in accordance with Organic Law 15/1999, of 13 December, on Personal Data Protection.

12.8. Documentation

The Investigator/site shall maintain the trial documents in accordance with ICH Topic E6 Section 8, and in compliance with the corresponding regulatory requirements.

The key documents shall be filed in accordance with GCP guidelines or for a longer period, if required by the applicable regulations.

The patients’ original study data (medical records) must be stored in accordance with the filing period applicable at the study sites, but for no less than 15 years.

13. TRANSLATIONAL STUDY

See Annex V to the protocol

14. REFERENCES

1. Crocetti E, Trama A, Stiller C, Caldarella A, Soffietti R, Jaal J et al. Epidemiology of glial and non-glial brain tumours in Europe. Eur J Cancer 2012; 48: 1532-1542.

2. Fuentes-Raspall R, Vilardell L, Perez-Bueno F, Joly C, Garcia-Gil M, Garcia-Velasco A et al. Population-based incidence and survival of central nervous system (CNS) malignancies in Girona (Spain) 1994-2005. J Neurooncol 2011; 101: 117-123.

3. Stupp R, Hegi ME, Mason WP, van den Bent MJ, Taphoorn MJ, Janzer RC et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. Lancet Oncol 2009; 10: 459-466.

4. Malkoun N, Chargari C, Forest F, Fotso MJ, Cartier L, Auberdiac P et al. Prolonged temozolomide for treatment of glioblastoma: preliminary clinical results and prognostic value of p53 overexpression. J Neurooncol 2012; 106: 127-133.

5. Roldan Urgoiti GB, Singh AD, Easaw JC. Extended adjuvant temozolomide for treatment of newly diagnosed glioblastoma multiforme. J Neurooncol 2012; 108: 173-177.

6. Groves MD, Jr., Plummer AB. Helping patients make the best decision regarding duration of temozolomide chemotherapy treatment. Continuum (Minneap Minn) 2012; 18: 421-425.

7. Brandsma D, Stalpers L, Taal W, Sminia P, van den Bent MJ. Clinical features, mechanisms, and management of pseudoprogression in malignant gliomas. Lancet Oncol 2008; 9: 453-461.

8. Hegi ME, Diserens AC, Gorlia T, Hamou MF, de Tribolet N, Weller M et al. MGMT gene silencing and benefit from temozolomide in glioblastoma. N Engl J Med 2005; 352: 997-1003.

9. Brandes AA, Tosoni A, Spagnolli F, Frezza G, Leonardi M, Calbucci F et al. Disease progression or pseudoprogression after concomitant radiochemotherapy treatment: pitfalls in neurooncology. Neuro Oncol 2008; 10: 361-367.

10. Stupp R, Tonn JC, Brada M, Pentheroudakis G. High-grade malignant glioma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol 2010; 21 Suppl 5: v190-193.

11. Mason WP, Maestro RD, Eisenstat D, Forsyth P, Fulton D, Laperriere N et al. Canadian recommendations for the treatment of glioblastoma multiforme. Curr Oncol 2007; 14: 110-117.

12. Malkoun N, Fotso MJ, Cartier L, Forest F, Auberdiac P, Chargari C et al. Benefit of a prolonged adjuvant treatment with temozolomide for the management of patients with glioblastoma. Cancer Radiother 2011; 15: 202-207.

13. American Society of Clinical Oncology editor. Gilbert MR, Wang M, Aldape KD, Stupp R, Hegi M, Jaeckle KA et al. RTOG 0525: A randomized phase III trial comparing standard adjuvant temozolomide (TMZ) with a dose-dense (dd) schedule in newly diagnosed glioblastoma (GBM). Proceedings of the 2011 ASCO Meeting. June 3-7. Chicago. USA.

14. Weller M, Stupp R, Reifenberger G, Brandes AA, van den Bent MJ, Wick W et al. MGMT promoter methylation in malignant gliomas: ready for personalized medicine? Nat Rev Neurol 2010; 6: 39-51.

15. Holdhoff M, Grossman SA. Controversies in the adjuvant therapy of high-grade gliomas. Oncologist 2011; 16: 351-358.

16. Franceschi E, Tosoni A, Brandes A. Adjuvant temozolomide: how long and how much? Expert Rev Anticancer Ther 2008; 8: 663-665

17. Balaña C, Vaz MA, Lopez D et al. Should we continue temozolomide beyond 6 cycles in the adjuvant treatment of glioblastoma without and evidence of clinical benefit? A cost analysis based on prescribing patterns in Spain. Clin Transl Oncology; 2013, (DOI) 10.1007/s12094-013-1068-3

Annex I. KARNOFSKY PERFORMANCE STATUS

CLINICAL AND FUNCTIONAL CONDITIONS	SCORE
Normal	100
Able to carry out normal activities	90
Minor signs or symptoms	80
Cares for self; unable to carry out normal activities or to do active work	70
Requires occasional assistance, but able to care for most of his/her needs	60
Requires assistance and frequent medical care	50
Disabled; requires special care and assistance	40
Severely disabled; hospitalisation indicated; death non-imminent	30
Moribund	10
Death	0

Annex II. BARTHEL INDEX

THE BARTHEL INDEX

Patient Name: _____
Rater Name: _____
Date: _____

Activity	Score
FEEDING 0 = unable 5 = needs help cutting, spreading butter, etc., or requires modified diet 10 = independent	_____
BATHING 0 = dependent 5 = independent (or in shower)	_____
GROOMING 0 = needs to help with personal care 5 = independent face/hair/teeth/shaving (implements provided)	_____
DRESSING 0 = dependent 5 = needs help but can do about half unaided 10 = independent (including buttons, zips, laces, etc.)	_____
BOWELS 0 = incontinent (or needs to be given enemas) 5 = occasional accident 10 = continent	_____
BLADDER 0 = incontinent, or catheterized and unable to manage alone 5 = occasional accident 10 = continent	_____
TOILET USE 0 = dependent 5 = needs some help, but can do something alone 10 = independent (on and off, dressing, wiping)	_____
TRANSFERS (BED TO CHAIR AND BACK) 0 = unable, no sitting balance 5 = major help (one or two people, physical), can sit 10 = minor help (verbal or physical) 15 = independent	_____
MOBILITY (ON LEVEL SURFACES) 0 = immobile or < 50 yards 5 = wheelchair independent, including corners, > 50 yards 10 = walks with help of one person (verbal or physical) > 50 yards 15 = independent (but may use any aid; for example, stick) > 50 yards	_____
STAIRS 0 = unable 5 = needs help (verbal, physical, carrying aid) 10 = independent	_____
TOTAL (0–100):	_____

Provided by the Internet Stroke Center — www.strokecenter.org

The Barthel ADL Index: Guidelines

1. The index should be used as a record of what a patient does, not as a record of what a patient could do.
2. The main aim is to establish degree of independence from any help, physical or verbal, however minor and for whatever reason.
3. The need for supervision renders the patient not independent.
4. A patient's performance should be established using the best available evidence. Asking the patient, friends/relatives and nurses are the usual sources, but direct observation and common sense are also important. However direct testing is not needed.
5. Usually the patient's performance over the preceding 24-48 hours is important, but occasionally longer periods will be relevant.
6. Middle categories imply that the patient supplies over 50 per cent of the effort.
7. Use of aids to be independent is allowed.

References

Mahoney FI, Barthel D. "Functional evaluation: the Barthel Index."
Maryland State Medical Journal 1965;14:56-61. Used with permission.

Loewen SC, Anderson BA. "Predictors of stroke outcome using objective measurement scales."
***Stroke.* 1990;21:78-81.**

Gresham GE, Phillips TF, Labi ML. "ADL status in stroke: relative merits of three standard indexes."
***Arch Phys Med Rehabil.* 1980;61:355-358.**

Collin C, Wade DT, Davies S, Horne V. "The Barthel ADL Index: a reliability study."
***Int Disability Study.*1988;10:61-63.**

Copyright Information

Annex III. MINI MENTAL STATE EXAMINATION MMSE

MINI MENTAL STATE EXAMINATION (MMSE)

Based on Folstein et al. (1975), Lobo et al. (1979)

Name: Male [ ] Female [ ]

Date: Date of birth: Age:

Studies/Profession: History no.:

Comments:

What is the year? 0-1 **What is the season? 0-1** **What is the date? 0-1** **What is the month? 0-1** **What is the day? 0-1**	**TEMPORAL ORIENTATION (Max. 5)**
Where are we (hospital or place)? 0-1 **What storey (or floor, room, department)? 0-1** **What town (city)? 0-1** **What province? 0-1** **What country (or nation, autonomous region)? 0-1**	**SPATIAL ORIENTATION (Max. 5)**
Name three objects Coin-Horse-Apple (or Ball-Flag-Tree), listing 1 per second. Then ask the patient to repeat them. The first repetition is used for scoring. Give 1 point for each correct answer. Then repeat them until the subject learns all 3, up to a maximum of 6 times. **Coin 0-1 Horse 0-1 Apple 0-1** **(Ball 0-1 Flag 0-1 Tree 0-1)**	**No. of necessary repetitions** **REGISTRATION-Immediate Recollection (Max. 3)**
If you have 30 coins and you give me three at a time, how many do you have left each time? Stop the test after 5 subtractions. If the subject is unable to complete the test, ask him/her to spell "WORLD" backwards. **30 0-1 27 0-1 24 0-1 21 0-1 18 0-1** **(O 0-1 D0-1 N0-1 U0-1 M0-1)**	**ATTENTION-CALCULATION (Max 5)**
Ask the subject to state the three words mentioned beforehand. **Coin 0-1 Horse 0-1 Apple 0-1** **(Ball 0-1 Flag 0-1 Tree 0-1)**	**Deferred RECOLLECTION - (Max 3)**
NAMING. **Show the subject a pencil or pen and ask him/her "What is this?". Do the same with a wristwatch. Pencil 0-1 Watch 0-1** REPETITION. **Ask the subject to repeat the phrase:** "No ifs, ands, or buts"**(or** "There were 5 dogs in a field") **0-1** COMMANDS. **Ask the subject to follow the command:** "Take the paper in your right hand, fold it in half, and put it on the floor". **Takes with right hand 0-1 folds in half 0-1 places on floor 0-1** READING. **Write** "Close your eyes" *clearly on a piece of paper.* **Ask the subject to read and obey the instruction 0-1** WRITING. **Ask the subject to write a sentence (with a noun and a verb) 0-1** COPYING. **Draw 2 intersecting pentagons and ask the subject to copy them. To score a point, all 10 angles must be present as well as the intersection. 0-1**	**LANGUAGE (Max. 9)**
Reference scores **27 or more: normal** **24 or more: suspected memory loss 12-24: impairment** **9-12: dementia**	**Total Score (Max.: 30 points)**

a.e.g.(1999)

Annex IV. RANO RESPONSE CRITERIA

	CR	PR	SD	PROGRESSION
T1-GD	**0**	**≥ 50% ↓**	**>50% ↓** **<25% ↑**	**≥25% ↑***
T2/FLAIR	**=, ↓**	**=, ↓**	**=, ↓**	**=, ↑***
NEW LESIONS	**0**	**0**	**0**	**0. +***
CORTICOSTEROIDS	**0**	**=, ↓**	**=, ↓**	**=, ↑***
SYMPTOMS	**=, ↑**	**=, ↑**	**=, ↑**	**=, ↓***
	all	**all**	**all**	**Any ***

COMPLETE RESPONSE:

- Disappearance of signal in T1GD MRI
- Stability or reduction in T2/FLAIR images
- No new radiological lesions
- Clinical stability or improvement
- Absence of corticosteroids or stable minimum dose

PARTIAL RESPONSE:

- 50% image reduction in the MRI with T1GD
- Stability or reduction in T2/FLAIR images
- No new radiological lesions
- Clinical stability or improvement
- With reduced or stable steroids

PROGRESSION: Any of the following

- 25% tumour increase in T1GD MRI
- Significant increase in T2/FLAIR images*
- Appearance of new lesions
- Irreversible neurological deterioration, even in the absence of radiological deterioration.
- Need to increase dexamethasone (for more than 2 weeks) to prevent progressive neurological deterioration.

NOTE: Only progression criteria 1, 2 and 3 are applicable for the purposes of the patient's enrolment in the clinical trial.

STABLE DISEASE:

- T1GD image with an increase of less than 25% or a decrease below 50%
- Stability in the T2/FLAIR image
- No new radiological lesions
- Clinically stable
- Stable cortisone doses (that do not need increasing to maintain neurological deterioration)

* not attributable to radiotherapy, demyelinating disease, ischemia, infection, seizures, post-operative changes or other treatment-related effects

Annex V. ASSOCIATED MOLECULAR SUB-STUDY

The efficacy of the most commonly used neuro-oncology drugs (nitrosoureas, temozolomide and procarbazine) stems from the chloroethylating or methylating lesion they produce on the DNA. The O6-methylguanine DNA methyltransferase (MGMT) repair enzyme repairs DNA by directly eliminating an alkyl group from the O6-guanine atom on the DNA of cells exposed to alkylating agents[31-33]. The gene that encodes said enzyme (MGMT) contains CpG dinucleotide islands that act as gene-coding promoters.[34] If said islands present methylation, the gene is silenced and no repair protein is produced. As such, the lesion produced by chemotherapy on the tumour cell becomes irreversible and the cell enters apoptosis. The efficacy of treatment with said drugs is thus better if there is no protein and, as such, if the MGMT gene presents promoter methylation.[35, 36]

TMZ is a second-generation imidazotetrazine derivative that spontaneously undergoes hydrolysis to the active metabolite under physiological conditions and acts as a DNA-methylating agent.

Due to their mechanism of action, it is known that cells with the capacity to repair methylated DNA to unmethylated DNA through, among other enzymes, their plentiful supply of O6-methylguanine DNA methyltransferase (MGMT), may overcome DNA lesions and, as such, prevent apoptosis. The enzyme is encoded by the MGMT gene. MGMT may be methylated at GpG islands and incorrectly transcribed, thereby causing enzyme inactivity.

The importance of the enzyme and its relationship with the response to alkylating or methylating agents in a variety of tumours is already understood.[31, 32] However, in the phase III EORTC study, which indicated temozolomide in first-line therapy, the MGMT methylation status was also determined in cases where there was a tumour sample, and it was established that said methylation status was a significant predictive factor of TMZ response. [35] However, it is yet to prove useful for distinguishing between patients and deciding upon a different course of treatment. During the DNA repair process, the enzyme is consumed and has to be resynthesised. As such, prolonged administration (in continuous or dose-dense regimens) may improve the drug's anti-tumour activity. [12]

On the basis of an international consensus, any study conducted on temozolomide must also be accompanied by a study on MGMT status in tumour cells.[37]

Other TMZ-resistant proteins have also been described (MSH1/MSH6), which will be analysed a posteriori in the Tissue Microarray.

The histological materials – in the form of the paraffin block containing the specimen from the initial surgery – will be sent to the Anatomy Department of the HUGTIP (Dr Carrato), where the histology will be confirmed and a baseline molecular study of the MGMT gene will be performed using the methylation-specific polymerase chain reaction (MSP) technique. (Dr Carolina Sanz). The MGMT result from the site of origin will also be accepted. These results will then be reported to the CRO for the centralised and stratified randomisation process, as well as subsequent statistical processing, and will also be forwarded to the patient's site of origin. Samples of tissue will be set aside to prepare a tissue microarray for subsequent immunohistochemistry studies on IDH1 and resistance proteins or TMZ sensitivity (MSH6, MSH2), and any future studies that may arise as a consequence of the trial results.

In the event of surplus materials, these will be returned to the site of origin.

Annex VI. DECLARATION OF HELSINKI

The latest version of the Declaration of Helsinki can be found at:

http://www.wma.net/en/30publications/10policies/b3/

Annex VII. NCI COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS V.4.0

Version 4.0 of the Common Terminology Criteria for Adverse Events (CTCAE) can be found at the following link:

http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

Annex VIII. TRIAL SCHEDULE

GEINO 14-01 study flowchart