Protocol summary

Title of the Study	A phase III trial of marizomib in combination with standard temozolomide-based radiochemotherapy versus standard temozolomide-based radiochemotherapy alone in patients with newly diagnosed glioblastoma - MIRAGE
Objective(s)	The primary objective of this study is to compare overall survival (OS) in patients receiving marizomib in combination with standard treatment (temozolomide (TMZ) with concomitant radiotherapy (RT), followed by TMZ maintenance therapy: TMZ/RT→TMZ) with patients receiving standard treatment only (TMZ/RT→TMZ). The testing strategy is defined to assess this objective in both the whole population and the subgroup of unmethylated MGMT (O-6-methylguanine-DNA methyltransferase) patients with adequate statistical power. Secondary objective is to compare progression free survival (PFS) in the two treatment arms in the whole population. Further secondary objectives are: • To assess the safety and tolerability of marizomib combined with TMZ/RT→TMZ. • To assess objective and self-perceived neurocognitive function and quality of life of patients treated with this approach. • Descriptive and correlative translational research. • To evaluate pharmacokinetics in the MRZ arm
Methodology	This is a multicenter, randomized, controlled, open label phase III superiority trial with an early stopping rule for futility. After signing the informed consent form and upon confirmation of the patient eligibility, patients will be randomized 1:1 to the experimental arm (addition of marizomib to the standard treatment) or the standard arm. Stratification factors are: Institution; Age (≤55, >55 years); Karnofsky performance status (70/80, 90/100); Extent of surgery (partial/open biopsy, gross total).
Number of patients Number planned (Statistical design) Number analyzed	For this study, we assumed that in the whole trial population, the standard treatment plus marizomib presents with a superior OS efficacy compared to the standard treatment alone estimated by a hazard ratio equal to 0.74 (26% reduction of the hazard of death). This corresponds to a median OS of 16 months in the standard treatment alone compared to 21.6 months for standard treatment plus MRZ. We also assume that at the time of final analysis, the MGMT methylation status will be distributed 60% unmethylated, 30% methylated and 10% undetermined. We also hypothesized that the MRZ effect would be mainly present in the unmethylated MGMT subgroup where it would display a HR=0.70 (median OS of 13 months in the control arm and compared to 18.6 months for marizomib). The effect in the methylated MGMT subgroup would be HR>0.80 and in the undetermined cases which are

	assumed to be a balanced mixture of unmethylated and methylated MGMT cases the effect would be in the line with the overall population, i.e. HR=0.74. Based on the network of institution, it should be possible to recruit 400 patients/year (150 patients in the first year, and then 400 patients per year). For the primary endpoint OS, the formal statistical testing is based on comparisons between two treatment groups in both ITT population and unmethylated subgroup. We will use a graphical method to control overall Type 1 error at one-sided 2.5%. We have to recruit 750 patients to show the OS difference with 86% power (taking ino account the interim analysis for futility) and overall one-sided 1.5% significance in the whole population and with 80.7% power and one sided 1% significance in the unmethylated MGMT subgroup (uMGMT). We will recruit these patients in about 30 months and will follow them up for about 19 months, the time necessary to observe the required 488 deaths (320 in uMGMT). We will perform the test in the ITT population and in the uMGMT subgroup simultaneously. If one of them is significant, we will attribute the assigned alpha to the other. At the final analysis, we will provide treatment effect estimates in the uMGMT and in the methylated MGMT (mMGMT) subgroups. In order to avoid exposing too many patients to a possibly ineffective and/or toxic treatment, a non-binding futility analysis will be conducted on the whole population.

Diagnosis and main criteria for inclusion	**Inclusion Criteria**
	
	• Histologically confirmed newly diagnosed glioblastoma (WHO grade IV)
	• Tumor resection (gross total or partial), or open biopsy only (no stereotactic biopsy)
	• Availability of FFPE tumor block or 24 unstained slides for MGMT analysis
	• Patient must be eligible for standard TMZ/RT→TMZ
	• Karnofsky performance score (KPS) ≥ 70
	• Recovered from effects of surgery, postoperative infection and other complications of surgery (if any)
	• The patient is at least 18 years of age on day of signing informed consent
	• Stable or decreasing dose of steroids for at least 1 week prior to inclusion
	• The patient has a life expectancy of at least 3 months
	• Patient has undergone a brain MRI within 14 days of randomization but after intervention (resection or biopsy)
	• The patient shows adequate organ functions as assessed by the specified laboratory values within 2 weeks prior to randomization defined as adequate bone marrow, renal and hepatic function within the following ranges:
	
	  • WBC ≥ 3×10^9/L
	  • ANC ≥ 1.5×10^9/L
	  • Platelet count of ≥ 100×10^9/L independent of transfusion

	• Hemoglobin ≥ 10 g/dl • Total Bilirubin ≤ 1.5 ULN • ALT, AST, alkaline phosphatase (ALP) ≤ 2.5 × ULN • Serum creatinine < 1.5 x ULN or creatinine clearance (CrCl) > 30 mL/min (using the Cockcroft-Gault formula) • Women of child bearing potential (WOCBP) must have a negative urine or serum pregnancy test within 7 days prior to randomization • Patients of childbearing / reproductive potential must agree to use adequate birth control measures, as defined by the investigator, during the study treatment period and for at least 6 months after the last study treatment. A highly effective method of birth control is defined as those which result in low failure rate (i.e. less than 1% per year) when used consistently and correctly. Patients must also agree not to donate sperm during the study and for 6 months after receiving the last dose of study treatment. • Women who are breast feeding must agree to discontinue nursing prior to the first dose of study treatment and until 6 months after the last study treatment. • Ability to take oral medication • Ability to understand the requirements of the study, ability to provide written informed consent and authorization of use and disclosure of protected health information, and agree to abide by the study restrictions and return for the required assessments. • Before patient registration/randomization, written informed consent must be given according to ICH/GCP, and national/local regulations. **Exclusion Criteria** • Patients with known IDH mutation (IDH mutation testing should be conducted for younger patients (<55years old), patients with tumors with atypical features, or with history or present concurrent lower grade gliomas. • Prior treatment for glioblastoma other than surgery; prior RT to brain and/or prior chemotherapy for lower grade glioma. Placement of BCNU wafer during surgery is not allowed Planned additional treatment with Tumor-Treating Fields • Known hypersensitivity to the active substance or any of the excipients in the IV formulation • History of thrombotic or hemorrhagic stroke or myocardial infarction in past 6 months • Congestive heart failure (New York Heart Association Class III to IV), symptomatic ischemia, conduction abnormalities uncontrolled by conventional intervention, and myocardial infarction within 6 months prior to first dose • Concurrent severe or uncontrolled medical disease (e.g., active systemic infection, diabetes, hypertension, coronary artery disease, psychiatric disorder) that, in the opinion of the investigator, would

	compromise the safety of the patient or compromise the ability of the patient to complete the study • Known history or current evidence of active Hepatitis B (e.g., positive HBV surface antigen) or C (e.g., HCV RNA [qualitative] is detected) • Known or current evidence of Human Immunodeficiency Virus (HIV) (positive HIV-1/2 antibodies) • Prior or second invasive malignancy, except non-melanoma skin cancer, completely resected cervical carcinoma in situ, low risk prostate cancer (cT1-2a N0 and Gleason score ≤ 6 and PSA < 10 ng/mL), either totally resected or irradiated with curative intent (with PSA of less than or equal to 0.1 ng/mL) or under active surveillance as per ESMO guidelines Other cancers for which the subject has completed potentially curative treatment more than 3 years prior to study entry are allowed. • Presence of any psychological, familial, sociological or geographical condition potentially hampering compliance with the study protocol and follow-up schedule; those conditions should be discussed with the patient before registration in the trial.
Treatment Test product, dose and mode of administration Duration of treatment	Experimental arm: Standard radiotherapy (RT)(60 Gy in 30 fractions over 6 weeks) + TMZ 75 mg/m^2 p.o. daily for 6 weeks (during radiotherapy) and marizomib (MRZ) dose 0.8 mg/m^2 IV at days 1, 8, 15, 29 and 36. This is followed, after 4-week break, by up to 6 cycles of maintenance TMZ 150-200 mg/m^2 p.o. on days 1-5 of a 28-day cycle and up to 18 cycles of maintenance MRZ treatment (0.8 mg/m^2 IV) at days 1, 8, 15 of a 28-day cycle until disease progression, unacceptable toxicity or withdrawal of consent. Standard arm: Standard radiotherapy (RT) (60 Gy in 30 fractions over 6 weeks) + TMZ 75 mg/m^2 p.o. daily for 6 weeks (during radiotherapy) then (after 4-week break) up to 6 cycles of maintenance TMZ 150-200 mg/m^2 p.o. on days 1-5 of a 28-day cycle. All patients will have regular follow-up by MRI, 4 weeks after the end of radiotherapy (RT), then every 8 weeks thereafter until progression. Once patients stop study treatment, they will be followed as per institution's standard.
Criteria for evaluation Efficacy Safety	Response and progression will be assessed by RANO criteria as determined by the local investigator. OS is defined as the number of days from date of randomization to the date of death due to any cause. If a patient has not died, the data will be censored at the last date documented to be alive. PFS is defined as the number of days from date of randomization to the date of earliest disease progression or to the date of death due to any cause, if disease progression does not occur. MMSE is a brief, standardized tool to grade patients' neurocognitive function. It is an 11-question measure that tests five areas of neurocognitive function: orientation, registration, attention and calculation, recall, and language.

	This study will use the International Common Terminology Criteria for Adverse Events (CTCAE), version 5.0, for adverse event reporting.
Statistical methods	We will conduct all efficacy analyses in the intent-to-treat (ITT) population. In the primary analyses, differences between the treatment groups in OS will be assessed by a stratified LogRank test adjusted for the stratification factors assessed at randomization (except institution). We consider this test as confirmatory and will perform it at a 1-sided significance level α=0.015 in the ITT population and α=0.01 in the uMGMT subgroup. If one of them is significant, the assigned alpha will be transferred to the other and the overall Type 1 error will be controlled at one-sided 2.5% by graphical method. Kaplan-Meier survival curves will be presented by treatment group, in the ITT population and by MGMT subgroup, together with a summary of associated statistics including the corresponding two-sided 95% confidence intervals. The hazard ratio (including two sided 97% and 95 % confidence interval in the ITT population and two sided 98% and 95 % confidence interval in the uMGMT subgroup) of the Marizomib group over the control group will be calculated by Cox's proportional hazards model stratified by the stratification factors assessed at randomization (except institution). A re-randomization-test analysis will be performed to support the primary analysis in the ITT population and in uMGMT. Secondary analyses of efficacy are supportive and will be analyzed in a non-confirmatory sense. Therefore no adjustments for multiplicity will be done. The following analyses will be performed. For PFS, the same analyses as for OS will be performed in the ITT population. For PFS and OS, Kaplan-Meier estimates will be calculated and unstratified Cox's proportional hazards model will be fit for various subgroups. Forest Plot will be displayed with a subgroup by treatment interaction test. The distribution of the MMSE at each time point of evaluation will be described on the two treatment arms separately using means and their associated standard error (a graphical display will be considered). Median and range will also be provided. The proportion of patients with 'normal' and 'impaired' MMSE score at baseline and at key timepoints of evaluation will also be displayed. Longitudinal analysis might also be performed and detailed in the SAP.

Translational research	FFPE tumor tissue will be prospectively collected for central testing of the MGMT status. Since MGMT status will be used in the primary OS analysis to determine if there is a difference in treatment effect which is dependent on this variable, this initial tumor tissue is a mandatory requirement within this protocol. We will also collect mandatory plasma and serum samples at study entry, after completion of RT and, every 3 cycles and at progression, as well as plasma samples for extracellular vesicle isolation (optional) for biomarker discovery. In this study, we plan to investigate the proteasomal activity in patients with sufficient amount of tissue available for these analyses. We have 2 objectives: firstly, we would like to confirm baseline proteasome activity and clinical response (PFS and OS), and secondly we want to see if the proteasome activity is modulated by marizomib in patients with tissue at baseline and at salvage surgery. To perform proteasome activity measurement, frozen tumor tissue, at study entry and after salvage surgery, should it occur, will be collected. Moreover, proteasomal activity will also be measured in PBMCs, collected at study entry, after completion of RT and, every 3 cycles and at progression. Future research might include isolation of ctDNA and gDNA from plasma. Depending on the study results, we may look for soluble factors in the serum which may be of interest as potential biomarkers. The ultimate goal of such work would be to define a tumor specific signature in the peripheral blood which could be used to monitor and predict response to therapy. Exploratory research may also include evaluation of a multigene signature to predict benefit from marizomib. FFPE samples will be stored at the EORTC BTG Tissue Repository (Rotterdam, NL) and blood derivates will be stored at the Intergrated BioBank Luxembourg (Luxembourg, LU).
Quality of Life	The main objective of QOL assessment within this trial is to determine the impact of addition of marizomib to temozolomide (TMZ) and radiation therapy (RT) on five chosen domains being primarily global QOL, with fatigue, physical function, neurocognitive function, communication and motor dysfunction as secondary QOL outcomes. It is expected that these are likely to be most affected in patients, based on the toxicity profiles and information of previous studies. Quality of life will be assessed through the EORTC Quality of Life Questionnaire (QLQ-C30) version 3 and the EORTC Brain Cancer module (QLQ-BN20), designed for use in brain tumor patients undergoing protocol treatment or radiotherapy. They will be collected every 16 weeks (± 7 days) until death, end of study, start of new anticancer treatment or lost to follow-up whichever comes first