1. PROTOCOL SYNOPSIS

Title of Study: Open-label, non-randomized, two-stage study to evaluate the efficacy, safety, pharmacokinetics and pharmacodynamic effects of PQR309 in patients with progressive glioblastoma

Protocol Number: PQR309-004

Development Phase: 2

Study Treatment:
- PQR309
- Capsules
- 20 mg, 80 mg
- Supplier: PIQUR Therapeutics AG, Basel

Background Information and Study Rationale:
Glioblastoma – World Health Organization (WHO) grade IV glioma - remains the most aggressive sub-type of glioma. Its incidence is estimated to be in the range of 3/100,000 worldwide. Despite the introduction of combined modality treatment with temozolomide chemoradiotherapy (TMZ/RT→TMZ), which improves survival for some patients, prognosis remains poor with a median survival in the range of 12-16 months. There is no standard systemic therapy available for recurrent or progressive glioblastoma. Salvage chemotherapy infrequently results in radiographic and/or clinical improvement and has a limited impact on overall survival (OS). Therapy with conventional and experimental agents for progressive glioblastoma is unsatisfactory and the proportion of these patients who are alive and progression-free at 6 months is in the range of 15-30% in contemporary clinical phase II trials, e.g., assessing nitrosourea- or bevacizumab-based regimens. Active, well tolerated agents that target the underlying molecular abnormalities in gliomas are urgently needed. The PI3 kinase (PI3K) and mammalian target of rapamycin (mTOR) pathway is activated in most glioblastomas and represents a potential therapeutic target. PQR309 is an oral, dual pan-PI3K and mTOR inhibitor that penetrates the blood-brain barrier at pharmacodynamically active concentrations. This study plans to evaluate PQR309 in treatment of patients with first progression of glioblastoma during or after TMZ/RT→TMZ.

Primary Objective:
To evaluate clinical efficacy of PQR309 in the treatment of progressive glioblastoma

Secondary Objective(s):
- Overall safety and tolerability of PQR309 in the treatment of progressive glioblastoma
- Overall clinical efficacy of PQR309
- Pharmacokinetics (PK) of PQR309 in plasma
- PQR309 concentration in tumor tissue, cerebrospinal fluid (CSF) and skin

Exploratory Objectives:
- Pharmacodynamic (PD) effects of PQR309 in tumor and skin tissue
- Correlation of PQR309 PK and its tissue concentration (tumor, CSF and skin) with PD effects
- Correlation of PQR309 PK, its tissue concentration (tumor, CSF and skin) and PD effects with clinical efficacy and safety endpoints

Primary Endpoint:
Progression-free survival rate at 6 months (PFS6) based on RANO criteria [30]

Secondary Endpoint(s):
- Incidence and severity of AEs and SAEs
- Changes in vital signs (pulse rate, blood pressure, body temperature), performance status (PS), physical examinations, body weight, ECG, PHQ-9 questionnaire and GAD-7 mood scale score
- Changes of routine laboratory assessments (blood chemistry, hematology,
Pharmacokinetics:
- PQR309 plasma concentration, and PK parameters: $C_{\text{max}}$, $t_{\text{max}}$, AUC$_{0-24}$, AUC$_{\text{last}}$, AUC$_{0-\infty}$, and $t_1/2$
- PQR309 concentration in tumor and CSF (only surgical cohort)
- PQR309 concentration in skin

Additional Clinical Efficacy based on RANO criteria:
- Overall Response Rate (ORR) including complete and partial response (CR and PR), duration of response, and progression-free survival at 3 months (PFS3)
- Changes in levels of phosphoproteins in tumor and skin tissue associated with inhibition of PI3K / mTOR and RAF-RAS-MAPK pathways, e.g. pAKT, pS6, p4EBP1, pERK etc. as compared with control tissue
- Mutational status of genes known to be relevant to glioblastoma (e.g. PTEN, PI3K, EGFR, etc.)
- Correlative evaluation of: clinical efficacy, PK, tumor tissue, CSF and skin concentration, PD and safety endpoints
- Overall survival (OS)

Study Design:
Open-label, non-randomized, two-stage, multi-center study evaluating clinical efficacy, safety, pharmacokinetics and pharmacodynamic effects of PQR309 in patients with progressive glioblastoma during or after standard temozolomide chemoradiotherapy (TMZ/RT→TMZ).

The first stage of the study will enroll a minimum of 18 patients with glioblastoma at first progression during or after TMZ/RT→TMZ, evaluable for the primary study objective, the rate of PFS according to RANO criteria at 6 months (PFS6). Following the completion of recruitment of patients in the first stage of the study, the decision will be made by the study team (study investigators and the sponsor), based on the continuous evaluation of safety and efficacy data, whether to continue recruitment of patients in the second stage while awaiting the data analyses (see the statistical evaluations below). 17 additional patients may be enrolled for the second stage of the study, for a minimum of 35 patients in total, evaluable for PFS6 analysis. All patients evaluable for the primary endpoint will be followed until disease progression or death.

Secondary objectives, PQR309 treatment safety and PK will be evaluated in all enrolled patients in both study stages.
In the surgical cohort (operable patients), PQR309 concentration in tumor tissue and CSF and its PD effects in tumor tissue will be evaluated following short preoperative administration of PQR309. Relevant PD parameters in tumor tissue obtained from patients administered 80 mg PQR309 p.o. once daily for three consecutive days prior to surgical resection will be compared to untreated control samples. Archival tumor tissue samples from first glioblastoma resections will serve as controls.
In addition, PQR309 concentration and its PD effects in skin as a surrogate tissue will be evaluated. To that end, skin biopsies will be collected at baseline and during surgery.
Surgical resection of the tumor will be performed on day 3 within 2-3 hours after the last PQR309 intake. During the surgical procedure, tumor tissue, CSF and skin will be collected in the same timeframe.
It is estimated that 10 evaluable patients administered PQR309 pre-operatively may be needed for the initial evaluation of PQR309 concentration in tumor tissue, CSF and skin and its PD effects in tumor tissue and skin.
Patients enrolled in the surgical cohort may be treated postoperatively with PQR309 with the fixed once daily dose of 80 mg p.o. after adequate recovery from surgery.
and will be included in the evaluation of the primary endpoint. Treatment with PQR309 may be initiated not earlier than 7 but not later than 14 days after surgery to minimize a delay in the treatment. These patients will be evaluated according to the same procedures as described for non-surgical cohort except for skin biopsies and PK assessments.

In the non-surgical cohort, patients will be continuously treated with the fixed once daily dose of 80 mg p.o. PQR309 concentration and its PD effects in skin as a surrogate tissue will be evaluated. To that end, skin biopsies will be collected at baseline and during treatment on Cycle 1 Day 15. In addition, archival tumor tissue samples from first glioblastoma resections will be collected for analysis.

It is estimated that 10 evaluable patients administered PQR309 continuously once daily at 80 mg p.o. may be needed for the initial evaluation of PQR309 concentration and its PD effects in skin.

All patients enrolled in the study may be treated with PQR309 until unacceptable AE, tumor progression, patient’s request for withdrawal, investigator judgment or death whichever comes first.

All patients treated with PQR309 will be followed for AEs 30 days after administration of the last dose of PQR309.

Study Population: 35 patients with a minimum of 18 patients to be enrolled in the first stage of the study.

Subject Selection: Inclusion Criteria:

1. Patients with histologically confirmed glioblastoma at first progression following or during standard temozolomide chemoradiotherapy (TMZ/RT→TMZ).
2. ≥ 18 years of age.
3. Radiographic demonstration of disease progression by RANO criteria.
4. Only for patients of the surgical cohort:
   - Eligible for open resection of progressive tumor according to standard practice of the study center
   - Availability of adequate surgical tissue sample for the evaluation of concentration of PQR309 in the tumor and its PD effect
   - Patients treated with PQR309 after incomplete surgical resection may still have measurable disease according to RANO criteria and may therefore be evaluable for evaluation of response to treatment with PQR309 according to RANO criteria. The best response in patients treated with PQR309 after complete surgical resection is stable disease. All patients can be assessed for PFS6.
5. Only for patients of the non-surgical cohort:
   - Presence of at least one lesion of bi-dimensionally measurable disease by MRI with a contrast-enhancing tumor of at least 1 cm (10 mm) in the longest diameter on baseline MRI is required for patients who do not undergo surgery at relapse. For patients who undergo surgery for recurrence, but do not participate in the presurgical PQR309 dosing cohort, the same rules regarding response assessment as in the surgical cohort apply. All patients can be assessed for PFS6.
6. Patient must have at least 1 formalin-fixed paraffin-embedded archival tumor tissue block representative of glioblastoma available from the first surgical resection of glioblastoma.
7. One prior systemic therapy regimen: patients must have received at least one dose of TMZ in the first line therapy. More than 6 cycles and alternative
dosing regiments of TMZ are allowed.

8. If receiving corticosteroids, patients must have been on a stable or decreasing dose of corticosteroids and no more than 8 mg dexamethasone equivalent for ≥ 5 days prior to baseline MRI.

9. Karnofsky Performance Score (KPS) ≥70%.

10. More than 12 weeks from radiotherapy (RT).

11. More than 4 weeks from last administration of TMZ.

12. More than 4 weeks from any investigational agent (at the judgment of the investigator and in agreement with lead investigator and PIQUR).

13. Adequate hematological, liver and renal function defined as follows:
   - absolute neutrophil count (ANC) ≥1.5x10⁹/l
   - platelets ≥ 100x10⁹/l
   - hemoglobin ≥ 100g/L
   - Total bilirubin ≤ 1.5 times the upper limit of normal (ULN)
   - Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≤ 2.5 times ULN
   - Serum Creatinine ≤ 1.5 times ULN.

14. Able and willing to swallow and retain oral medication.

15. Female and male patients of reproductive potential must agree to use effective contraception from screening until 90 days after discontinuing study treatment.

16. Willing and able to sign the informed consent and to comply with the protocol for the duration of the study.

Exclusion Criteria:

1. Second or later glioblastoma relapse.

2. Received more than one systemic treatment regimen for glioblastoma.

3. Patients receiving enzyme-inducing anti-epileptic drug (EIAED) within 7 days of the first dose of PQR309.

4. Patient is taking a drug with known risk to promote QT prolongation and Torsades de Pointes.

5. Patient is currently using herbal preparations or medications. Patient should stop using herbal medications 7 days prior to the first dose of the study drug.

6. Patients with glioblastoma known to contain IDH1 or 2 mutation.

7. Other concomitant anti-tumor therapy as determined by the study team.

8. Prior treatment with intracerebral agents, e.g. proilityprospan 20 with carmustine wafer.

9. Patients unable to undergo contrast-enhanced MRI.

10. Fasting glucose > 7.0 mmol/L or HbA1c > 6.4%.

11. Medically documented history of or active major depressive episode, bipolar disorder (I or II), obsessive-compulsive disorder, schizophrenia, a history of suicidal attempt or ideation, or homicidal ideation (e.g. risk of doing harm to self or others), or patients with active severe personality disorders.


13. Patient has an uncontrolled intercurrent illness, including but not limited to, ongoing or active infection, known HIV infection, chronic liver disease, chronic renal disease, pancreatitis, chronic pulmonary disease, active cardiac disease or cardiac dysfunction, interstitial lung disease, active autoimmune disease, uncontrolled diabetes, neuropsychiatric or social situations that would limit compliance with the study requirements.

14. Presence of gastrointestinal disease or any other condition that could interfere significantly with the absorption of the study drug.

15. Women who are pregnant or breast feeding.
16. Women able to conceive and unwilling to practice an effective method of birth control from screening until 90 days after discontinuing study treatment (women of childbearing potential must have a negative urine or serum pregnancy test within 7 days prior to first dose of PQR309).

### Study Treatment Administration

**Surgical cohort:** Pre-operative administration of 80 mg PQR309 p.o. once daily for 3 consecutive days prior to surgical resection of the tumor. On Day 3, the day of surgery, PQR309 must be taken 1 h prior to anesthesia premedication, 2-3 h prior to tumor removal during the surgery.

Should they consent and be eligible, patients enrolled in the surgical cohort may be treated postoperatively with PQR309 with the fixed once daily dose of 80 mg p.o. after adequate recovery from the surgery (7-14 days, as judged by responsible investigator at the study site) following the same procedures as for the non-surgical cohort.

**Non-surgical cohort:** Treatment of non-operable patients with progressive glioblastoma: 80 mg PQR309 p.o. once daily until unacceptable AE, tumor progression, patient’s request for withdrawal, investigator judgment or death whichever comes first.

### Dose modifications, delays, discontinuation

Dose modifications and delays will be performed as outlined in the protocol. Patients in the surgical cohort are required to receive all 3 consecutive pre-surgical PQR309 doses to be evaluable. Patients who require a treatment delay of >14 days due to AE despite dose reductions as specified in the protocol (Table 2), will be withdrawn from the trial. However, if in the investigator’s opinion a patient may benefit from re-starting treatment this can be considered after discussion with the sponsor.

Patients will be discontinued from trial treatment in case of any of the following reasons:

- Clinical or symptomatic or radiographic disease progression or relapse
- Unacceptable toxicity related to the study drug (as assessed by the treating physician or the sponsor)
- Patient voluntary withdrawal
- Investigator decision
- Non-compliance to protocol
- Pregnancy
- Termination of the study by the sponsor

In addition to the above reasons that require discontinuation, other circumstances may necessitate discontinuation of a subject from the study, as judged by the investigator or sponsor.

Patients may voluntarily withdraw from the study at any time for any reason.

### Study Assessments

#### Efficacy assessments:

Tumor response evaluation according to RANO criteria and performed every 8 weeks (± 7 days) after start of treatment and until objective disease progression.

#### Safety assessments:

Continuous monitoring and recording of AEs and SAEs (AEs will be graded according to NCI-CTC AE, version 4.03)

Measurement of biochemistry, hematology, coagulation, and urinalysis variables; measurement of vital signs, physical examination, KPS evaluation, ECG, PHQ-9 questionnaire and GAD-7 mood scale score (see Schedules of Assessment - Table 4 and Table 5).

#### PK assessments:
Surgical cohort
- On Day 1, blood samples (6 ml) for PK analysis will be collected at: pre-dose, and 0.5h, 1h, 2h, 4h, 6h and 8h post-dose.
- On Day 2, a blood sample (6 ml) for PK analysis will be collected at pre-dose (=24h post first dose).
- On Day 3, the day of surgery, blood samples (6 ml) for PK analysis will be collected: at pre-dose, and 0.5h, 1h, 2h, 4h, 6h and 8h post-dose.
- On Day 4, a blood sample (6 ml) for PK analysis will be collected at 24h after the last dose given on Day 3.

Non-surgical Cohort
- On Cycle 1 Day 1, blood samples (6 ml) for PK analysis will be collected at: pre-dose, and 0.5h, 1h, 2h, 4h, 6h and 8h post-dose.
- On Cycle 1 Day 2, a blood sample (6 ml) for PK analysis will be collected pre-dose (=24h post first dose).
- On Cycle 1 Day 8, blood samples (6 ml) for PK analysis will be collected 1h pre-dose and 1h post-dose.
- On Cycle 1 Day 15, blood samples (6 ml) for PK analysis will be collected at pre-dose, and 0.5h, 1h, 2h, 4h, 6h and 8h post-dose.
- On Cycle 2 Day 1, blood samples (6 ml) for PK analysis will be collected: pre-dose and 1h post-dose

Exploratory assessments
PD effects and concentration of PQR309 in tumor tissue, CSF and skin.
In the surgical cohort, adequate aliquots of surgical resection tissue will be snap-frozen for PK analyses and formalin-fixed for PD analyses. Skin biopsies will be collected at baseline for patients in both cohorts. In the surgical cohort, an additional skin biopsy will be collected during surgery in the same time frame as tumor tissue and CSF collection. In the non-surgical cohort, an on-treatment skin biopsy will be collected on Cycle 1 Day 15, two hours post PQR309 administration.

Statistical Methods

**Statistical methods**
Definition of populations for analysis:
The intent-to-treat (ITT) analysis set will comprise all subjects who receive ≥ 1 dose of PQR309. For the surgical cohort this refers to patients who have received ≥ 1 dose of PQR309 following surgery. The ITT analysis set will be used in all analyses.

**Primary endpoint**
The sample size for the evaluation of the primary endpoint of this two-stage study is based on the criteria selected for the futility (Stage 1) and positive efficacy (Stage 2) applying Simon's two-stage design [41].
The sample size of a 35 patients is chosen in order to test, whether the observed PFS6 indicates activity of the PQR309 treatment. We consider a PFS6 of 10% or less to be clinically unimportant and would like to have reasonable power to detect a PFS6 of 30% or more.
The proportion of patients with progressive glioblastoma treated with conventional and experimental agents who are alive and progression-free at 6 months is in the range of 15-30% in contemporary clinical phase II trials, e.g. assessing nitrosourea- or bevacizumab-based regimens. PFS6 of at least 20% is considered a clinically meaningful effect for the initial evaluation of PQR309 treatment effect in this patient population.
The null hypothesis for the proposed Simon's two-stage design is that the true PFS6 based on RANO criteria is 10%, which will be tested against a one-sided alternative (30%).
In the first stage, a minimum of 18 evaluable patients will be needed for analyses. If among these 18 patients, only 2 patients or less (10%) are free of progression and alive at 6 months, futility criteria are met and the null hypothesis will be accepted. Following completion of the recruitment of patients in the first stage of the study, the decision will be made by the study team (study investigators and the sponsor) based on continuous evaluation of safety and efficacy data, whether to continue further recruitment of patients in the second stage while awaiting the data analyses. 17 additional patients may be enrolled for the second stage of the study, to a minimum of 35 patients in total, evaluable for PFS6 analysis. The null hypothesis will be rejected, if among a total of 35 evaluable patients, 7 patients or more (20%) are free of progression and alive at 6 months. This approach yields a type I error rate of 0.05 and power of 0.9 when the true PFS6 is 30%.

PQR309 PD effects and tumor tissue concentration should be evaluated in 10 patients. This sample size is chosen as a practical number to reasonably evaluate the PQR309 PD effect; no explicit sample size justification is made.

**Secondary endpoint**

All demographic and baseline characteristics will be descriptively summarized. Categorical variables will be summarized as the number and percentage of patients in each category. Continuous variables will be summarized as mean, standard deviation, median, minimum, and maximum.

Safety variables include the reported AEs, laboratory tests, vital signs, KPS performance status, physical examination, PHQ-9 questionnaire score and GAD-7 mood scale score.

AEs will be coded and evaluated for severity using NCI-CTCAE, version 4.03 and will be summarized by system organ class and preferred term. Separate summaries will be generated for the following:

- All AEs
- Severe AEs (Grade 3 or higher)

Listings will be provided of:

- SAEs
- AEs leading to treatment discontinuation
- AEs resulting in death
- AEs listed according to maximum severity

The frequency of AEs will be tabulated by grade across all cycles and for each cycle of treatment. All patients who receive any study treatment will be considered evaluable for safety.

**Laboratory Tests:** Summary statistics for baseline, each post-baseline measurement, and change from baseline for each post-baseline measurement will be presented for each hematology, serum chemistry, liver function test, electrolyte, and urinalysis parameter.

**Vital Signs:** Summary statistics for baseline, each post-baseline measurement, and change from baseline for each post-baseline measurement will be presented for each vital sign parameter.

**PHQ-9 questionnaire and GAD-7 mood scale scores:** Summary statistics for baseline, each post-baseline measurement and change from baseline for each post-baseline measurement will be presented for each score.

**KPS:** Number and percent of patients having each KPS score will be presented for baseline and each post-baseline measurement.

**Physical Exams:** Data from physical exams will be presented in the data listings.

**Pharmacokinetics:** PK parameters of PQR309 including; $t_{\text{max}}$, $C_{\text{max}}$, $AUC_{0-24}$, $AUC_{\text{last}}$, $AUC_{0-\infty}$, $t_{1/2}$, as well as its concentration in tumor tissue and skin will be
evaluated and presented using descriptive statistical methods.

**Exploratory endpoints:** Exploratory correlative analyses will be used to correlate PQR309 PD effects, with PQR309 PK and clinical endpoints such as OS.

PK, PD, tumor and CSF concentration analysis data sets will include data from all subjects, who have necessary baseline and on-study measurements to provide interpretable results for the specific parameters of interest.