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LSTA1-GBM-2A: study protocol for an exploratory phase 2a randomised controlled trial evaluating tumour-homing peptide certepetide with temozolomide in glioblastoma multiforme

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ABSTRACT

Introduction Glioblastoma multiforme (GBM) is an aggressive primary brain tumour associated with a poor prognosis despite standard-of-care treatment, including surgical resection, radiotherapy and temozolomide (TMZ) chemotherapy. Certepetide (also known as LSTA1, CEND-1) is an investigational tumour-penetrating peptide that facilitates the extravascular delivery and intratumoural penetration of co-administered immune/chemotherapeutics; however, it has not yet been evaluated in clinical trials for the treatment of intracranial malignancies.

Methods and analysis LSTA1-GBM-2A is an exploratory phase 2a, double-blind, placebo-controlled, randomised, proof-of-concept investigator-initiated trial assessing the safety, tolerability and preliminary efficacy of certepetide in combination with standard-of-care TMZ, compared with TMZ with a matching placebo, in subjects with newly diagnosed GBM.

The trial is funded by Lisata Therapeutics, sponsored by Tartu University Hospital and conducted at hospitals in Estonia and Latvia. Subjects are randomised in a 2:1 ratio. Following initial surgery and radiotherapy with concurrent TMZ, the subjects receive intravenous certepetide or placebo alongside six cycles of adjuvant TMZ treatment. The primary endpoint is overall survival. The target number of subjects is 30. The first subject was recruited in January 2024, and accrual is ongoing.

Ethics and dissemination This study was approved by the Republic of Estonia State Agency of Medicines (17 October 2023) and the State Agency of Medicines of the Republic of Latvia (1 February 2024). The results of this study will be published in peer-reviewed journals and reported at academic conferences.

Trial registration number 2023-506813-23-00.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ LSTA1-GBM-2A is a phase 2a, double-blind, placebo-controlled, randomised, proof-of-concept trial being conducted in Estonia and Latvia.
⇒ The trial design includes a safety lead-in phase and randomisation stratified by O6-methylguanine-DNA methyltransferase (MGMT) promoter hypermethylation status.
⇒ Disease progression is assessed by two blinded radiologists according to modified Response Assessment in Neuro-Oncology criteria.
⇒ Given the exploratory nature of the trial, the small sample size limits the interpretability of efficacy outcomes.

INTRODUCTION

Glioblastoma multiforme

Glioblastoma multiforme (GBM) is the most frequent malignant primary brain tumour in adults. Its incidence is approximately 3 per 100 000 people per year without major regional variation. GBM represents approximately 15% of all brain tumours and accounts for 50% of gliomas.[1]

The diagnosis of GBM is currently based on imaging techniques, histology and molecular–genetic profiling of tumour tissue.[2] According to the fifth edition of the WHO Classification of Tumours of the Central Nervous System, isocitrate dehydrogenase (IDH)-wildtype glioblastoma lacks mutations in the IDH1, IDH2 and H3 genes.[3] GBM, IDH-wildtype should be diagnosed in the setting of an IDH-wildtype diffuse and astrocytic glioma in adults if there is microvascular proliferation, necrosis, telomerase reverse transcriptase (TERT) promoter mutation, epidermal growth factor receptor (EGFR) gene amplification, or +7/−10 chromosome copy number changes.[3]

The current standard of care for GBM consists of maximal safe surgical resection followed by concurrent chemoradiotherapy and maintenance chemotherapy with temozolomide (TMZ). Despite this multimodal approach, the median overall survival (mOS) remains approximately 15 months,[4 5] and the 5-year survival rate is less than 5%.[6] GBM is highly resistant to existing therapies and is characterised by a high rate of recurrence. Given the dismal prognosis, the development of novel treatments is of utmost importance.

Temozolomide

TMZ has been widely used as the standard chemotherapy for newly diagnosed GBM[7] since the landmark publication by Stupp and colleagues demonstrated that the addition of TMZ chemotherapy to radiation led to a 2-month increase in mOS,[4 5] which is one of the most significant enhancements in GBM survival achieved by a novel chemotherapeutic drug to date.

In accordance with the European Medicines Agency authorisation[8] TMZ is indicated for adult patients with newly diagnosed GBM in combination with focal radiotherapy (concomitant phase) followed by up to six cycles of TMZ monotherapy (monotherapy phase).

Although TMZ is widely used in clinical practice, the pharmacogenomic characteristics of its pharmacokinetics and pharmacodynamics in patients with GBM have not been definitively elucidated. Genetic polymorphisms involving the O6-methylguanine-DNA methyltransferase (MGMT), mismatch repair (MMR), base excision repair (BER), homologous recombination repair/nonhomologous end-joining (HRR/NHEJ), ATP binding cassette subfamily B member 1 (ABCB1) and acid phosphatase 7 (ACP7) genes may affect the therapeutic effect of TMZ.[9–11]

Certepetide

Certepetide, an internalising RGD (arginylglycylaspartic acid or iRGD), cyclic peptide, is an investigational drug developed for use with other cancer therapeutics for the treatment of patients with solid tumours. Certepetide (also known as CEND-1 or LSTA1) was originally developed by CEND Therapeutics. In September 2022, CEND Therapeutics merged with Caladrius Biosciences to form Lisata Therapeutics.

Certepetide is a disulphide-bridged cyclic peptide (sequence CRGDKGPDC) that homes to and penetrates a variety of solid tumours. Its tumour specificity is mediated by a three-step mechanism involving two classes of cell surface receptors and a tumour-derived protease, which together trigger an endocytic uptake pathway that enhances intratumoural accumulation of co-administered and conjugated anticancer therapeutics.[12 13]

Mechanistically, the integrin-binding RGD motif of certepetide first binds to αvβ3 and αvβ5 integrins on tumour endothelium and tumour cells.[14] These integrins are upregulated in tumours but not in normal tissues,[14] enabling the peptide to accumulate specifically in tumour vessels and stroma. The second step involves proteolytic cleavage after the second basic (K) residue, producing a truncated peptide (CRGDK) that exposes a C-terminal CendR motif (R/KXXR/K). This motif binds neuropilin-1 (NRP-1), a multiligand cell surface receptor involved in vascular and neurobiology, ligand uptake and tumour progression.[15] Binding of CRGDK to NRP-1 activates the CendR endocytic pathway, which enhances tumour delivery of conjugated and co-administered payloads.[13 15 16] Both αvβ3/αvβ5 integrins and NRP-1 are overexpressed in GBM, making the CendR pathway a promising route for targeted delivery in GBM therapy.[17 18] The CendR endocytic vesicles can accommodate payloads ranging from small molecules to nanoparticles. Remarkably, the CendR system can transport payloads co-administered with certepetide even when they are not chemically conjugated to it.[12 19–21] This co-administration mode offers a significant advantage by eliminating the need for chemical modification of anticancer drugs to enable deep tumour penetration. Consequently, the efficacy of any approved anticancer drug could be improved with certepetide without creating new chemical entities. Moreover, unlike conventional targeting strategies, the drug-carrying capacity of this co-administration approach is not limited by the number or availability of receptors for the homing peptide.[22]

Preclinical in vivo studies using certepetide in mouse tumour models, including human tumour xenografts, have demonstrated strong tumour homing activity and improved survival.[12 13 21 23–29]

Clinical trials

Two clinical trials on the effects of certepetide in advanced solid tumours have been completed to date. Several clinical trials have published preliminary data.

The CEND1-001 study was a phase 1b/2a open label, multicentre, dose escalation, safety, pharmacokinetic, pharmacodynamic and efficacy study of certepetide, administered alone and in combination with nab-paclitaxel and gemcitabine in 31 patients with metastatic pancreatic ductal adenocarcinoma.[30] The trial's safety and preliminary efficacy results were promising. No certepetide dose-limiting toxicities were observed in the safety population, the objective response rate (ORR) was 59%, and the mOS was 13.2 months versus 8.5 months historical control standard of care nab-paclitaxel and gemcitabine data. Certepetide injection (CEND1-201) in patients with advanced metastatic pancreatic ductal adenocarcinoma is a phase 1b/2a clinical trial that was conducted in China.[31] The results revealed an acceptable safety profile of certepetide combined with gemcitabine and locally produced nab-paclitaxel in patients with metastatic pancreatic ductal adenocarcinoma. Compared with historical standard of care data, the subjects included in the trial had an improved ORR of 42.9% versus 23% and an mOS of 11.1 months versus 8.5 months.

A safety and early efficacy study of certepetide in combination with durvalumab, gemcitabine and nab-paclitaxel,

as first-line treatment in locally advanced pancreatic ductal adenocarcinoma (iLSTA) is a phase 1 trial conducted in Australia, which has provisional results available, demonstrating both tolerability and efficacy.[32]

The ASCEND study: gemcitabine and nab-paclitaxel with certepetide or placebo in patients with untreated metastatic pancreatic ductal adenocarcinoma (ASCEND) is a phase 2 trial being conducted in Australia and New Zealand.[33] The preliminary data from the first cohort showed safety and a possible signal of benefit for efficacy. The patients receiving certepetide in combination with gemcitabine and nab-paclitaxel had an mOS of 12.42 months, whereas the group only receiving standard-of-care gemcitabine and nab-paclitaxel had an mOS of 9.72 months. In a sample of 95 patients, 4 complete responses were observed in the certepetide group, whereas 0 were observed in the standard-of-care group.

The safety data for certepetide suggest a favourable benefit–risk profile. The absence of any certepetide-related serious adverse events (SAEs) when administered as monotherapy and the low frequency of SAEs in combination with chemotherapy support the continued evaluation of this investigational drug.

METHODS AND ANALYSIS

Study design

LSTA1-GBM-2A is an exploratory phase 2a, double-blind, placebo-controlled, randomised, proof-of-concept investigator-initiated trial assessing the safety, tolerability and preliminary efficacy of certepetide in combination with standard-of-care TMZ, compared with TMZ with a matching placebo, in subjects with newly diagnosed GBM.

The trial consists of a safety lead-in phase involving the first three subjects followed by a randomised phase with the subsequent 27 subjects.

The safety lead-in phase

As a safety precaution, since certepetide had never been administered to subjects with intracranial malignancy, the first three subjects were not randomised. After successfully completing screening, the three subjects received certepetide on days 1–3 (run-in) to assess the safety of certepetide without concomitant administration of TMZ, and after 7 days, they received TMZ with certepetide on days 1 and 2 of the first two treatment cycles. TMZ monotherapy was administered on days 3–5 of cycles 1 and 2 and on days 1–5 for cycles 3–6 (figure 1).

After three subjects completed two treatment cycles, the data were evaluated based on predefined safety criteria:

- No treatment-related deaths (Common Terminology Criteria for Adverse Events (CTCAE) Grade 5)[34]
- No treatment-related life-threatening toxicities (CTCAE Grade 4)
- Fewer than three of the subjects develop a dose-limiting toxicity (DLT) requiring TMZ dose reduction.

If two of the subjects were to have developed a DLT (eg, DLT-qualifying CTCAE Grade 3 or higher adverse events) requiring TMZ dose reduction, the study would have proceeded but safety would have been reviewed after six subjects had been randomised and completed cycle 1.

At the time of writing this article, the safety lead-in phase was successfully completed. The predefined safety criteria were met, and the study continued without modification with the subsequent subjects being randomised to the main treatment arms.

The subjects participating in the safety lead-in were offered to continue participating in the trial according to the main study schema for the remaining treatment cycles.

The main study phase

The main study phase consists of a screening period, a run-in period, a treatment period, an end-of-treatment follow-up visit and a long-term follow-up period (figure 2).

Subjects who provide informed consent are screened for eligibility within 28 days prior to beginning the study treatment run-in phase. Once eligibility is confirmed, the subjects are randomised 2:1 to one of the two treatment groups (ie, TMZ+certepetide vs TMZ+matching certepetide-placebo).

During the 3 day run-in, subjects receive certepetide or matching placebo components of their randomised treatment regimen to assess the safety of certepetide/placebo; they do not receive TMZ. Cycle 1 of treatment commences 7 days after the start of the run-in. The subjects receive six cycles of treatment.

During treatment, study visits are scheduled every 4 weeks.

Radiological tumour assessments with gadolinium-enhanced MRI are regularly performed. Patient-reported outcomes are deployed. Laboratory evaluations are performed prior to treatment initiation and at 2-week intervals during treatment.

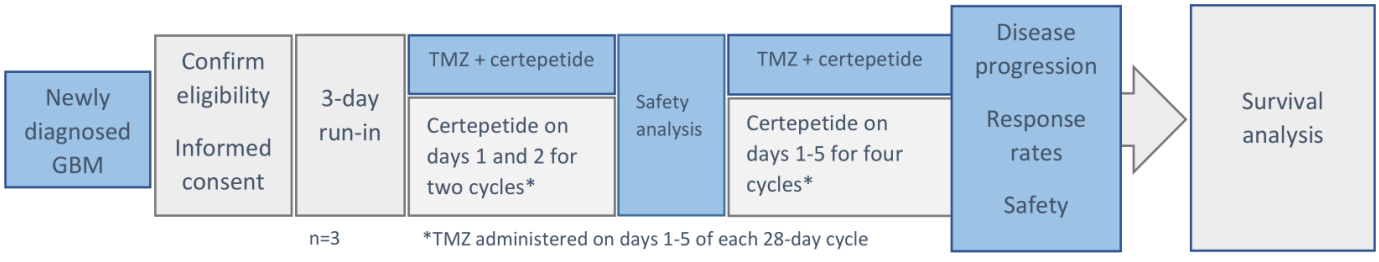

Figure 1 Safety lead-in schema. GBM, glioblastoma multiforme; TMZ, temozolomide.

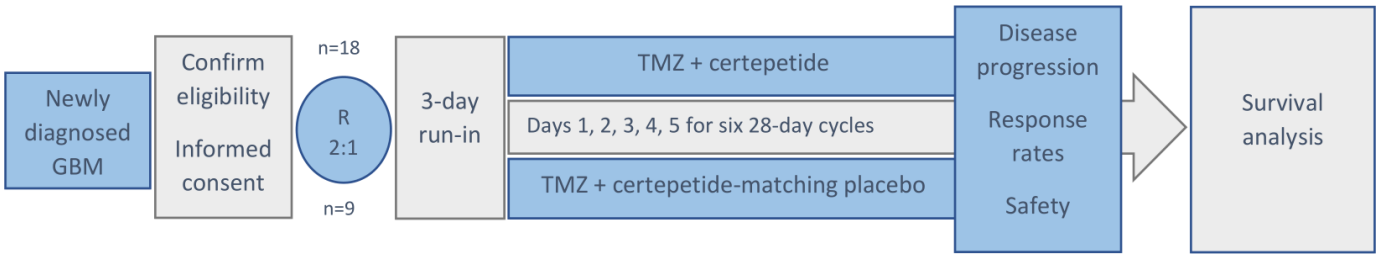

Figure 2 Main study schema. GBM, glioblastoma multiforme; R, randomisation; TMZ, temozolomide.

Ongoing safety, survival and subsequent anticancer therapies are assessed during long-term follow-up.

This trial is reported according to the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials)[35] checklist (online supplemental file 1).

Participating sites

Recruitment is ongoing at Tartu University Hospital, Estonia, North Estonian Medical Centre, Estonia and Riga East University Hospital, Latvia.

Estimated trial duration

Due to slower-than-expected subject accrual, the trial is planned to finish recruitment in 2026. The end of the study will occur when all of the subjects have completed 24 months of survival follow-up, died, withdrawn consent or been lost to follow-up, whichever occurs first, or when the study is terminated.

Inclusion criteria

Subjects who meet all the following criteria are eligible for this study:

1. Subjects must be ≥18 and < 81 years of age at time of screening and provide a written informed consent.
2. Subjects must have newly diagnosed and histologically confirmed intracranial GBM according to the 2021 WHO classification.
3. Subjects must have undergone primary surgical resection for GBM followed by the initial standard radiotherapy regimen (54–60 Gy/30 fractions) in combination with TMZ for 6 weeks.
4. Subjects must have sufficient time for recovery from prior surgery (at least 4 weeks).
5. Subjects must be able to undergo serial MRIs (CT may not be a substitute for MRI).
6. Eastern Cooperative Oncology Group performance status of 0, 1 or 2.
7. Life expectancy ≥3 months, as determined by the investigator.
8. Patients may have, or continue to receive corticosteroids, but she/he must be on a stable or decreasing dose for at least 14 days prior to randomisation (first dose of study drug for safety lead-in patients).
9. Subjects must have adequate organ and marrow function as defined by the following laboratory values obtained within 14 days prior to randomisation (first dose of study drug for safety lead-in patients):
   a. Platelets≥100×10^9/L (>100 000 per mm^3).
   b. White blood cell count≥3×10^9/L.
   c. Absolute neutrophil count≥1.5×10^9/L (1500 cells/mm^3).
   d. Serum albumin≥25 g/L.
   e. ALT and AST≤2.5 × upper limit normal (ULN).
   f. Bilirubin≤1.5 × ULN.
   g. Haemoglobin≥90 g/L. Patients may be transfused to meet this criterion.
   h. International normalised ratio<1.5 (for patients not receiving therapeutic anticoagulation).
   i. Serum creatinine≤1.5 × ULN or creatinine clearance ≥30 mL/min (if calculated using the Cockcroft-Gault formula below:)
      i. Female creatinine clearance = (140 − age in years) × weight in kg × 0.85/72 × serum creatinine in mg/dL.
      ii. Male creatinine clearance = (140 − age in years) × weight in kg × 1.00/72 × serum creatinine in mg/dL.
10. Adequate respiratory and cardiac function (partial pressure of oxygen in arterial blood ≥60 mmHg or oxygen saturation ≥92% on room air, and 12-lead ECG with normal tracing or clinically insignificant changes that do not require medical intervention).
11. Adequate contraception:
    a. All female patients will be considered to be women of childbearing potential (WOCBP) unless they are postmenopausal. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause or surgical sterilisation. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. WOCBP must agree to use a highly effective contraceptive measure to avoid pregnancy starting from recruitment and for at least 6 months following the last administration of the study drug. Highly effective birth control methods include combined hormonal contraception (oral, intravaginal, transdermal), progestogen-only hormonal contraception (oral, injectable, implantable), an intrauterine device, an intrauterine hormone-releasing system, bilateral tubal occlusion, a vasectomised partner and sexual abstinence. WOCBP must also agree to not donate ova/oocytes or freeze/store them for their own use for the purpose of reproduction during the treatment phase and 6 months after the last dose of study intervention.

b. Male patients and their female partners, who are WOCBP and are not practising total abstinence, must agree to condoms during the study and for a period of 3 months following the last administration of the study drug. A male participant must agree not to donate sperm for the purpose of reproduction during the study and for a minimum of 3 months after receiving the last dose of study intervention.

In the substantially amended protocol V.1.4 (protocol date dated 1 October 2024), the upper limit for age in the inclusion criteria was increased from <76 to < 81 years, and the screening duration was increased from 14 days to 28 days to improve recruitment.

Exclusion criteria

A subject who meets any of the following criteria will be excluded from the study:

1. Besides initial TMZ therapy, patients with prior cytotoxic or non-cytotoxic drug therapy or experimental drug therapy including chemotherapy, hormonal therapy or immunotherapy for the brain tumour.
2. Patients who received prior Gliadel wafers.
3. Patients with concurrent stereotactic radiosurgery or brachytherapy.
4. Patients with extracranial metastatic disease.
5. Patients with leptomeningeal dissemination.
6. Progression of GBM during or after the standard-of-care radiotherapy and TMZ 6-week regimen.
7. Subject has evidence of acute intracranial or intratumoural haemorrhage >Grade 1 by MRI. Subjects with resolving haemorrhage changes, punctate haemorrhage or hemosiderin may enter the study.
8. Any condition or comorbidity that, in the opinion of the investigator, would interfere with evaluation of study treatment or interpretation of patient safety or study results, including but not limited to:
   a. Any major surgery within 4 weeks of baseline.
   b. History of interstitial lung disease.
   c. Known acute or chronic pancreatitis.
   d. Active infection, including tuberculosis (TB). Clinical evaluation includes clinical history, physical examination and radiographic findings and TB testing in line with local practice, hepatitis B virus (known positive HBV surface antigen (HBsAg) result), and hepatitis C. Patients with a past or resolved HBV infection (defined as the presence of hepatitis B core antibody and absence of HBsAg) are eligible. Patients positive for hepatitis C virus (HCV) antibody are eligible only if PCR is negative for HCV RNA.
   e. HIV infection.
   f. Any other active infection (viral, fungal or bacterial) requiring systemic therapy.
   g. History of allogeneic tissue/solid organ transplant.
   h. Diagnosis of immunodeficiency.
   i. Has another diagnosis of cancer malignancy (except surgically excised non-melanoma skin cancer or carcinoma in situ of the cervix, or treated early-stage prostate cancer, or a malignancy diagnosed ≥5 years previously with no current evidence of disease and no therapy within 2 years prior to enrolment in this study).
   j. Clinically significant or symptomatic cardiovascular/cerebrovascular disease (including myocardial infarction, unstable angina, symptomatic congestive heart failure, serious uncontrolled cardiac arrhythmia) within 6 months before enrolment.
   k. Is pregnant or breastfeeding or planning to become pregnant.
9. Participation in another interventional clinical study within the last 1 year.
10. Patient has known hypersensitivity to TMZ or compounds with similar chemical composition to TMZ.
11. Patient with any significant history of non-compliance with medical regimens or with inability to grant reliable informed consent.
12. Patient who has been incarcerated or involuntarily institutionalised by court order or by the authorities.

Concomitant therapy

Patients should receive full supportive and palliative care (eg, pain control) as clinically indicated during the trial (including transfusion of blood products and analgesics) when appropriate.

Not-permitted medications:

1. Growth factors for neutropenia should not be used within 24 hours of planned TMZ administration.
2. Erythropoietin may not be used.
3. Other investigational drugs.

Surgical procedures for tumour debulking, other types of chemotherapy, immunotherapy or biological therapy must not be used. Further, additional stereotactic boost radiotherapy is not allowed. If any of these treatments are required, the patient will have to come off protocol therapy.

Recruitment

The subjects are recruited by investigators at the study sites.

Screening

Screening information, including clinical evaluation, laboratory assessments, tumour biomarkers, imaging, archival tumour tissue if available and pregnancy test for potential subjects is recorded in the electronic case report form, including reasons for ineligibility.

Randomisation

In the main phase of the trial after completing the safety lead-in phase, once eligibility is confirmed, subjects are randomised 2:1 to one of the two treatment groups (ie, TMZ+certepetide vs TMZ+matching certepetide-placebo) via block randomisation. The randomisation is stratified by MGMT promoter hypermethylation status. Separate lists of blocks of 3 were generated for each stratum (hypermethylated vs not hypermethylated) prior to the

start of the study using the electronic sealed envelope system.[36] Subjects included in the trial are assigned the group (certepetide or placebo) in the order of their inclusion in the study according to the appropriate list (hypermethylated vs not hypermethylated).

Blinding

LSTA1-GBM-2A is a double-blind study.

The treatment codes are available to an assigned unblinded pharmacist, who is involved in preparing the study drug for administration.

Drug administration

The two treatment arms of the main phase of the trial:

Treatment group 1: certepetide 3.2 mg/kg in combination with TMZ 150 or 200 mg/m^2 (orally) on days 1, 2, 3, 4 and 5 every 28 (+0…7) days for six cycles.

Treatment group 2: matching certepetide placebo in combination with TMZ 150 mg/m^2 (or 200 mg/m^2) (orally) on days 1, 2, 3, 4 and 5 every 28 (+0…7) days for six cycles.

Sequence of administration: TMZ is administered orally at a dose of 150 mg/m^2 in cycles 1 and 2 and 150 or 200 mg/m^2 in cycles 3–6, depending on tolerability. Immediately following TMZ administration, certepetide (3.2 mg/kg) or a matching certepetide placebo will be administered.

Dose modifications of TMZ are allowed to manage toxicity. Every effort should be made to provide maximal supportive therapy prior to implementing a dose reduction. The dose of certepetide will not be adjusted.

Study visits and assessments

Study visits and assessments are provided in table 1.

Tumour imaging

Progression, as per the modified Response Assessment in Neuro-Oncology criteria,[37] is assessed independently by two blinded radiologists in the study site country. If assessments differ, MRI studies will be reviewed in a multidisciplinary tumour board meeting.

To differentiate pseudoprogression from true disease progression, the initial imaging demonstrating glioblastoma progression will be designated as ‘preliminary progressive disease’, with study treatment continuing until a confirmatory scan is performed 4–8 weeks later.

When progression is confirmed by two radiologists, the patient will stop receiving study treatment and the patient’s further treatment will be discussed at a multidisciplinary tumour board meeting. Radiological tumour assessments are performed every 8 weeks (±7 days) for the first 24 weeks after randomisation, and every 12 weeks thereafter or when clinically indicated for up to 2 years after randomisation (first dose of study drug for safety lead-in subjects, who were not randomised). Tumour assessments will not be performed after confirmed disease progression or if a new anticancer therapy is commenced.

End of follow-up

A subject’s participation in the study follow-up will end for any of the following reasons: the subject is known to have died, consent is withdrawn for any further contact with study personnel, sponsor/Independent Ethics Committee/Regulatory Authority termination or suspension of the study, and the subject is lost to follow-up. Follow-up is capped at 2 years after the last patient is randomised.

Data collection and data management

An electronic data capture system is used for data collection and query handling. Case report forms, including data validation checks and change confirmations, were created in Research Electronic Data Capture (REDCap) prior to the start of the study.

Monitoring and safety

On-site monitoring and central monitoring are conducted by the Clinical Research Centre of Tartu University Hospital, Estonia. Data management is conducted by the sponsor, according to the study-specific Data Management Plan.

In accordance with the European Medicines Agency guidelines, an independent Data Safety Monitoring Board (DSMB) was installed. The DSMB conducted an interim assessment of the non-randomised safety lead-in cohort and will continue ongoing data monitoring to ensure participant safety throughout the trial.

Primary efficacy objective and endpoint

The primary efficacy objective is to determine the effect of certepetide in combination with TMZ on survival relative to matching certepetide placebo in combination with TMZ in patients with newly diagnosed GBM. The corresponding endpoint is OS, which is defined as median time from randomisation until death due to any cause.

Secondary efficacy objectives and endpoints

Progression-free survival (PFS) and duration of response (DoR) will be assessed as secondary endpoints of primary interest. We will also assess 6-month PFS and 12-month PFS.

To determine the effect of certepetide on milestone survival endpoints, we use 12-month survival and 24-month survival as endpoints.

To determine the effect of certepetide on the response rate, we will assess the ORR and disease control rate (DCR).

Quality of life assessments (European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire Core 30) will be used to determine any impact of certepetide in combination with TMZ on quality-of-life measures.

Safety endpoints

The incidence and severity of AEs are classified by CTCAE guidelines[34] and recorded. The frequency of Grade≥3 treatment-related AEs, the frequency of treatment-related

Table 1 Study visits and assessments

			Protocol treatment phase			Follow-up	
Study flow	**Screening***	**72-hour run-in**	**Day 1 of every cycle (every 4 weeks+0…7 days)**	**Day 15 of every cycle (±1 day)**	**Day 1 of cycle 3 and 5†**	**End of treatment‡**	**Every 12 weeks (±2 weeks)**
Sign informed consent	X						
Past medical history§	X						
Prior cancer therapy¶	X						
Concomitant medications	X		X		X	X	X
Current steroid dosage	X		X		X	X	X
Complete physical examination including neurological examination**	X	X††	X		X	X	X
Height and weight‡‡	X	X	X		X	X	X
Vital signs§§	X	X¶¶	X¶¶		X¶¶	X	X
12-lead ECG	X		X		X		
ECOG rating	X		X		X	X	X
Pregnancy test***	X		X		X	X	X
Urinalysis	X						
Clinical chemistry†††‡‡‡§§§	X		X		X	X	
Complete blood count‡‡‡§§§	X		X	X	X	X	
HIV, HBV, HCV and TB testing¶¶¶	X						
PT/PTT/INR‡‡‡§§§	X		X		X		
TMZ administration****			X		X		
Certepetide or placebo administration****		X††††	X		X		
Tumour assessment, gadolinium-enhanced MRI of the brain‡‡‡‡	X				X	X	X
AE assessment§§§§	X	X	X		X	X	
PROs¶¶¶¶	X				X	X	X
Pharmacokinetic evaluation*****					X		
Pharmacogenetic sampling†††††			X				
CtDNA sampling‡‡‡‡‡			X		X		
Disease progression§§§§§							X
Overall survival¶¶¶¶¶							X

Continued

Table 1 Continued

*Subjects who provide informed consent will be screened for eligibility within 28 days prior to beginning the study treatment run-in phase.
†In addition to assessments conducted every 4 weeks (+0…7 days), patient-reported outcomes will be deployed and gadolinium-enhanced MRI of the brain will be performed.
‡All patients who stop study treatment should have an end of treatment visit for assessment of adverse events 30 days (±3 days) after the last dose of study treatment.
§Medical history includes previous major medical history (eg, diabetes, cardiovascular disease, major chronic diseases and corresponding treatments, and previous surgical history (eg, diagnostic or therapeutic invasive surgery).
¶Includes histological diagnosis, pathological classification, clinical staging, genetic mutation results (if applicable) and treatment history.
**Physical examination: a thorough physical examination should be performed which includes examination of the head, eyes, neck, heart, lungs, abdomen, joints, extremities and skin. A neurological examination should also be performed, including: a mental status examination, speech, cranial nerves, muscle strength and tone, reflexes, coordination, sensory function and gait (if possible).
††Perform a targeted physical examination (does not need to be completed if performed less than 72 hours before randomisation or first dose of study drug for safety lead-in patients).
‡‡Height and weight: height to be measured only at screening.
§§Includes temperature, blood pressure, pulse rate and respiratory rate.
¶¶Perform a vital sign assessment pre-dose (0–120 min prior) and post-dose (within 15 min) including temperature, blood pressure, pulse rate and respiratory rate.
*** To be performed on all women of childbearing potential within 72 hours prior to dosing (day 1 of every cycle). Pregnancy testing will be conducted monthly on women of childbearing potential during follow-up in the first 6 months after the end of treatment visit.
††† Clinical chemistry includes sodium, potassium, chloride, magnesium, calcium, glucose, creatinine, urea, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, albumin, total protein, total bilirubin, lactate dehydrogenase, C-reactive protein.
‡‡‡Must be performed within 72 hours prior to dosing on day 1 of every cycle.
§§§Tests may be performed more frequently if clinically indicated.
¶¶¶TB testing if indicated. HIV, HBV and HCV testing if not performed in the past 3 months.
****To be administered on days 1, 2, 3, 4 and 5 every 28 days for six cycles in patients not part of the safety lead-in.
††††Administer certepetide or certepetide-matching placebo on days 1, 2 and 3 of run-in.
‡‡‡‡Gadolinium-enhanced MRI of the brain will be conducted during screening if not performed in the last 28 days before the planned randomisation visit (first dose of study drug for safety lead-in patients). Gadolinium-enhanced MRI of the brain will be conducted every 8 weeks during the treatment phase and every 12 weeks during the follow-up phase up to 2 years after randomisation (first dose of study drug for safety lead-in patients).
§§§§Adverse events according to Common Terminology Criteria for Adverse Events.
¶¶¶¶EORTC QLQ C-30 to be completed by patients. Also deployed before cycle 1 day 1.
*****Plasma sample collection for pharmacokinetic analysis of certepetide and TMZ will be conducted during cycle three at time points specified in the pharmacokinetic sampling plan.
†††††Pharmacogenetic sampling will be conducted only in cycle 1.
‡‡‡‡‡CtDNA sampling will be conducted on cycle 1 day 1 and cycle 3 day 1. ctDNA sampling will also be conducted when disease progression is confirmed.
§§§§§Patients are monitored for disease progression until confirmed disease progression or the commencement of a subsequent line of treatment, whichever occurs first.
¶¶¶¶¶May be conducted by telephone or based on medical record review.
AE, adverse event; ctDNA, circulating-tumour DNA; ECOG, Eastern Cooperative Oncology Group; EORTC QLQ C-30, European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire Core 30; HBV, hepatitis B virus; HCV, hepatitis C virus; INR, international normalised ratio; PROs, patient-reported outcomes; PT, prothrombin time; PTT, partial thromboplastin time; TB, tuberculosis; TMZ, temozolomide.

SAEs and the frequency of AEs of special interest will be used as endpoints.

The mean TMZ dose delivered per cycle, the mean TMZ dose over three cycles and over six cycles and the mean number of TMZ dose modifications (ie, dose delays, interruptions and discontinuations per cycle) will be used as endpoints to evaluate the impact on TMZ dose when administered in combination with certepetide.

Exploratory endpoints

Plasma samples will be collected and pharmacokinetic analysis of certepetide and TMZ will be conducted to characterise the pharmacokinetics of both drugs when administered in combination.

An exploratory pharmacogenetic analysis will be conducted to evaluate the possible associations of genomic polymorphisms with TMZ blood concentrations in GBM subjects receiving TMZ in combination with certepetide or placebo.

Circulating tumour DNA will be collected at specified time points and correlated with clinical outcomes.

Where available, exploratory tissue biomarkers will be evaluated from pretreatment archival tissue.

Statistical analyses

Descriptive statistics will be used to summarise the efficacy, safety and pharmacokinetic data from this study and with hypothesis testing performed for the primary and other selected efficacy endpoints.

For primary efficacy analysis, Kaplan-Meier survival curves will be constructed. Survival curves will be compared with the log-rank test. Treatment group median survival time will be estimated and compared with that of the placebo group by estimating the ratio of the estimated median survival time and its 95% CI. Cox regression analysis will be used to estimate HR.

12-month survival rates, 24-month survival rates, ORR and DCR will be compared across treatment groups using Fisher's exact test. Treatment group PFS and DoR will be compared with those in the placebo group with log-rank tests. Cox regression analysis will be used to estimate HR. Median PFS and DoR times will be estimated and compared with those in the placebo group by estimating the ratio of the estimated median survival time and its 95% CI.

Any statistical tests performed will be two-sided with an alpha level of 0.05. All analyses will be subject to formal verification procedures. Specifically, results will be verified using independent programming prior to the issuance of the draft statistical report. All documents will be verified by the lead statistician to ensure accuracy and consistency of analyses.

The efficacy analysis set will include all subjects randomised to study drug treatment group who received any amount of the study drug and completed a post-treatment tumour assessment and/or discontinued due to documented clinical progression. Subjects part of the safety lead-in will not be included in the efficacy analysis set.

All subjects who receive any amount of study drug will be included in the final summaries and listings of safety data.

Detailed information collected for each AE will include description of the event, duration, whether the AE was serious, intensity, relationship to study drug, action taken and clinical outcome. Intensity (severity) of the AEs will be graded according to the CTCAE.

A summary table will present the number of subjects observed with AEs and corresponding percentages. The denominator used to calculate incidence percentages consists of subjects receiving any amount of the study drug. Within each table, the AEs will be categorised by MedDRA (Medical Dictionary for Regulatory Activities) body system and preferred term. Additional subcategories will be based on event intensity and relationship to study drug.

Deaths and other SAEs will be tabulated.

A summary table will be prepared to examine the distribution of laboratory measures and vital signs over time.

Concentration-time profiles will be constructed from the obtained plasma pharmacokinetic samples. Maximum concentration and apparent time of maximum concentration will be determined. Non-compartmental analysis will be used to calculate area under the curve. A non-linear model will be used to estimate non-linear elimination parameters and volume of distribution.

Pharmacodynamic analyses will be summarised using descriptive statistics, if applicable. Subject disposition, demographics and baseline clinical characteristics will be presented for all randomised subjects. For each treatment group, the following will be presented: the number of subjects who meet all eligibility criteria, the number of subjects included in each analysis set, the number of subjects who completed the study and discontinued from the study and the reasons for early discontinuation at any point. The number of subjects dosed will be presented and the number of days on study treatment will be summarised for all treated subjects. The number of dose interruptions, modifications and discontinuations of all anticancer medication will be summarised.

Sample size

Formal sample size calculations were not required for this phase 2a, placebo-controlled, proof-of-concept trial, which is exploratory by design.

The planned sample size is 30. Three subjects have completed the safety lead-in, and 27 additional subjects will be randomised 2:1. 18 subjects will receive the study drug and 9 will receive the placebo.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

ETHICS AND DISSEMINATION

This study was approved by the Republic of Estonia State Agency of Medicines (17 October 2023, ethics approval reference number RKU-4/41) and the State Agency of Medicines of the Republic of Latvia (1 February 2024, ethics approval reference number 1-47/19), EUCT number 2023-506813-23-00. As of writing this article, V.1.4 (dated 1 October 2024, approved on 12 February 2025) is the latest approved substantially amended version of the study protocol. In accordance with European Union Clinical Trials Regulation no 536/2014, the application process of the clinical trial took place via the Clinical Trial Information System.

All patients provide written informed consent prior to being enrolled in the study (online supplemental file 2).

The study is conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines of the International Council for Harmonisation.

The results of this study will be published in peer-reviewed journals and reported at academic conferences.

DISCUSSION

Although multiple preclinical studies indicate that certepetide has tumour-homing properties in vitro and in vivo, its safety, tolerability and augmented efficacy when combined with standard of care chemotherapy must be established in clinical trials.

This trial is the first clinical trial to investigate the effects of certepetide for the treatment of an intracranial malignancy and the first in which certepetide is administered on consecutive days.

The LSTA1-GBM-2A trial has multiple strengths. As a randomised controlled trial, it employs hard, clinically relevant endpoints to assess treatment effects and identify signals related to both preliminary efficacy and safety. On completion, the trial will yield a cohort with comprehensive clinical documentation and archival tissue samples, providing a valuable resource for exploratory biomarker research and translational studies.

If the LSTA1-GBM-2A trial demonstrates positive outcomes, it could pave the way for a larger-scale study with greater statistical power to confirm the findings. Given the poor prognosis of GBM with the current standard of care treatment and the scarcity of effective treatment options, the development of a novel therapeutic agent would address a significant unmet medical need. Ultimately, our goal is to improve the standard of care for patients with GBM.

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Competing interests KKB and WS are employees and shareholders of Lisata Therapeutics, Inc. TT is an inventor on patents related to iRGDs and CendR, and a shareholder of Lisata Therapeutics, Inc. L-TK is the principal investigator of the current trial funded by Lisata Therapeutics, Inc. None of the other authors have conflicts of interest to declare.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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