

Stanford Cancer Institute

A National Cancer Institute
Comprehensive Cancer Center



A Phase IIa Study Assessing QBS72S For Treating Brain Metastases

IRB-63041

OnCore BRN0051

NCT05305365

IND-158711

Version 9: 31-May-2024**Initial SRC Approval Date: 22 October 2021 (for version dated 22 October 2021)**

Single-Center

Stanford Cancer Center
Stanford Medicine at Stanford University
875 Blake Wilbur Dr
Stanford Medicine
Stanford, CA 94305

Stanford Medicine IND-holder, Principal Investigator, and Protocol Director

Melanie Hayden Gephart, MD, MAS
300 Pasteur Drive
Stanford Medicine at Stanford University
Stanford, CA 94305
Phone: 650-497-7777
e-mail: mghayden@stanford.edu

Stanford Cancer Institute Sub-Investigators:

Michael Iv, MD
Stanford University Radiology
300 Pasteur Drive
Stanford, CA 94305
650-725-5384
miv@stanford.edu

Seema Nagpal, MD
Stanford University Neuro Oncology
300 Pasteur Drive
Stanford, CA 94305
650-725-8630
snagpal@stanford.edu

Additional Sub-Investigators:

Michael Lim, MD
Gordon Li, MD
Steven Chang, MD
Lawrence Shuer, MD

Lawrence Recht, MD
Reena Thomas, MD
Melinda Telli, MD
Michelle Melisko, MD

Biostatistician:

Summer Han, PhD
Stanford University Neurosurgery
1070 Arastradero Road
Palo Alto, CA 94304
650-724-5997
summer.han@stanford.edu

Study Coordinator:

Monica Granucci
Stanford SCI-CTO, CNS CRG
800 Welch Road
Stanford, CA 94305
650-388-8906
migranucci@stanford.edu

Regulatory Information

Study Agent:	QBS72S
IRB of Record:	Administrative Panels on Human Subjects in Medical Research; ("Stanford University IRB")
IND of record:	IND 158711, held by Dr. Hayden Gephart
Number of sites:	Single-center (Stanford Medicine at Stanford University)
IND Cross-reference:	IND 146107
Funding Sources:	California Breast Cancer Research Grant, NCI SBIR grant

STATEMENT OF COMPLIANCE

LEAD INVESTIGATOR STATEMENT

I have read and agree to the study, as detailed by this protocol document. I am aware of my responsibilities as an Investigator pursuant to the clinical trial protocol, the guidelines of [Good Clinical Practice \(GCP\)](#) ¹, the Declaration of Helsinki ², and the applicable Code of Federal Regulations (CFR) at [Title 21](#) ³ and [Title 45 Part 46](#) ⁴, as well as my responsibilities as a Lead Investigator, as a ClinicalTrials.gov Responsible Party under [42 CFR Part 11](#) ⁵ as promulgated pursuant to Section 801 of the Food and Drug Administration Amendments Act (FDAAA 801), and other applicable requirements of the participating institutions including the Stanford Cancer Institute, the Stanford Hospitals and Clinics, Lucile Packard Children's Hospital, and/or the Stanford University Medical Center. I agree to conduct the trial according to these regulations, guidelines, and requirements, and to appropriately direct and assist the participating staff under my authority, and that all staff members are aware of, and trained in, their clinical trial responsibilities.

All key study personnel have completed Human Subjects Protection Training.

Site Principal Investigators are expected to assure that no deviation from, or changes to the protocol, will take place without documented approval from the Institutional Review Board (IRB) of record, except where necessary to eliminate an immediate hazard(s) to the trial participants.

Lead Investigator's Name: Dr. Melanie Hayden Gephart
Name of Site: Stanford Cancer Institute
Stanford Medicine
Stanford, California

Principal Investigator's Signature: _____ **Date:** _____

- 1 FDA Guidance for Industry: current revision of the International Conference on Harmonization (ICH) Good Clinical Practice Guidelines E6.
- 2 World Medical Association, Declaration of Helsinki Ethical Principles for Medical Research Involving Human Patients.
- 3 United States Code of Federal Regulations (CFR), [Title 21, "Food and Drugs."](#)
- 4 CFR, [Title 45 "Public Welfare," Part 46 "Protection of Human Subjects."](#)
- 5 CFR, [Title 42 "Public Health," Part 11 "Clinical Trials Registration and Results Information Submission."](#)

Table of Contents

PROTOCOL SYNOPSIS.....	9
PROTOCOL SCHEMA.....	13
List OF ABBREVIATIONS & TERMS	14
1. INTRODUCTION	15
1.1. Study Rationale	15
1.2. Background	16
1.2.1. Overview of Study Agent	16
1.2.2. Mechanism of Action for QBS72S.....	16
1.2.3. Non-clinical Background	17
1.2.4. Clinical Background	23
1.2.5. Importance of the Clinical Trial.....	24
1.3. Potential Risks and Benefits	25
1.3.1. Known Potential Risks of Study Drug QBS72S	25
1.3.2. Known Potential Risks of Study Procedures	26
1.3.3. Known Potential Benefits	26
2. STUDY DESIGN, OBJECTIVES, AND OUTCOMES	27
2.1. Study Design.....	27
2.1.1. Overall Design	27
2.1.2. Primary Purpose of the Study	27
2.1.3. Study Cohorts	27
2.1.4. Scientific Justification for Study Design.....	27
2.1.5. Treatment Assignment.....	28
2.1.6. Justification for Dose.....	28
2.1.7. Study Timeline	28
2.1.8. End of Study Definition	28
2.2. Objectives	29
2.2.1. Primary Objective	29
2.2.2. Secondary Objectives	29
2.2.3. Exploratory Objectives	29
2.3. ClinicalTrials.gov Registration, Outcomes, and Results.....	29
2.3.1. Data Sharing Statement.....	29
2.3.2. Outcome Measures for ClinicalTrials.gov Results Reporting.....	30

3. PARTICIPANT SELECTION	31
3.1. Eligibility Criteria and Participant Eligibility Checklist	31
3.1.1. Inclusion Criteria	32
3.1.2. Exclusion Criteria	35
3.1.3. Lifestyle Considerations	37
3.1.4. Screen Failures	37
3.2. Recruitment and Retention Procedures	37
3.2.1. Recruitment	37
3.3. Informed Consent Process	38
3.3.1. Non-English Speakers	38
3.3.2. Physical Limitations to Consent	38
3.3.3. Cognitive Limitations to Consent	39
3.4. Registration and Enrollment	39
3.4.1. Participant Registration Procedures	39
3.4.2. Participant Enrollment Procedures	39
4. STUDY INTERVENTION	39
4.1. Investigational Drug	39
4.1.1. Name	40
4.1.2. Administration	40
4.1.3. Dose	40
4.1.4. Regulatory Status of QBS72S	40
4.1.5. QBS72S Preparation, Handling, Storage, and Accountability	41
4.1.6. Investigational Agent Supply	41
5. TREATMENT PLAN	42
5.1. Treatment Assignment	42
5.2. Treatment Dose	42
5.3. Treatment Schedule	42
5.4. Treatment Administration Restrictions	42
5.5. Treatment Duration	43
5.6. Dose Modifications and Dose-Limiting Toxicities	43
5.6.1. Dose Discontinuation	43
5.6.2. Dose Interruptions and Delays	43
5.6.3. Potential Adverse Events	44
5.7. Concomitant Medications, Procedures, and Supportive Care Guidelines	46
5.7.1. Premedication Guidelines	47

5.7.2.	Supportive Care	47
5.7.3.	Hematopoietic Growth Factors	47
5.7.4.	Corticosteroids	48
5.7.5.	Permitted Medications	48
5.7.6.	Prohibited Concomitant Medications	48
5.8.	Criteria for Removing Participants from Study Intervention and/or Study	49
5.8.1.	Withdrawal of Consent	49
5.8.2.	Lost to Follow-Up	50
5.9.	Duration of Follow-Up	50
5.10.	Study Completion or Discontinuation	50
6.	STUDY PROCEDURES AND ASSESSMENTS	52
6.1.	Schedule of Events	52
6.2.	Description of Procedures and Assessments	54
6.2.1.	Efficacy Assessments	54
6.2.2.	Safety and Other Assessments	55
7.	ADVERSE EVENTS AND REPORTING PROCEDURES	59
7.1.	Adverse Event Definitions	59
7.2.	Classification of Adverse Events	60
7.2.1.	Severity of Event	60
7.2.2.	Adverse Event Attribution to Intervention or Study	60
7.2.3.	Expectedness of Event	61
7.2.4.	Time Period of Event Assessment and Follow-Up	61
7.3.	Potential Adverse Events and Risks	61
7.4.	Adverse Event Monitoring and Collection	61
7.4.1.	Case Report Forms for Adverse Event Reporting	61
7.5.	Adverse Events of Special Consideration	62
7.5.1.	Progressive Disease	62
7.6.	Adverse Event Reporting	62
7.7.	Adverse Event Records	62
8.	CORRELATIVE AND SPECIAL STUDIES	63
8.1.	Sample Coding for Privacy Protection	63
8.2.	Pharmacokinetics (PKs)	63
8.2.1.	PK Background	63
8.2.2.	PK Collection and Handling	63
8.2.3.	Sites Performing Correlative Study	63

8.3.	Correlative Study of Biomarkers	64
8.3.1.	Biomarkers Background.....	64
8.3.2.	Biomarker Specimen Collection and Handling	64
8.4.	Correlative Study of Archival Tumor Tissue Specimens	64
8.4.1.	Archival Tumor Biopsy Background	64
8.4.2.	Archival Tissue Collection and Handling	64
8.4.3.	Archival Tissue Analysis	65
9.	REGULATORY CONSIDERATIONS AND DATA REPORTING	65
9.1.	Review and Approval of Protocol by IRB and SCI SRC	65
9.2.	Protocol Compliance and Deviations	65
9.2.1.	Compliance with the Protocol.....	65
9.2.2.	Protocol Deviations	65
9.3.	Data and Safety Monitoring Plan	66
9.3.1.	Monitoring.....	66
9.4.	Data Management.....	66
9.4.1.	Data Management Plan	66
9.5.	FDA Oversight.....	67
9.5.1.	Investigational New Drug (IND) Application Considerations	67
10.	STATISTICAL CONSIDERATIONS.....	68
10.1.	Statistical Plan.....	68
10.1.1.	Sample Size Determination	68
10.1.2.	Minimization of Bias	68
10.2.	Study Endpoints	69
10.2.1.	Primary Objective	69
10.2.2.	Secondary Objectives	73
10.2.3.	Exploratory Objectives	74
10.3.	Interim Analysis	76
10.4.	Primary Analysis.....	76
10.4.1.	Primary Analysis Population	76
10.4.2.	Primary Analysis Plan	76
10.5.	Secondary Analysis.....	77
10.5.1.	Secondary Analysis Population	77
10.5.2.	Secondary Analysis Plan.....	78
10.6.	Exploratory Analysis.....	79
10.6.1.	Analysis Population for Exploratory Objectives.....	79

10.6.2.	Analysis Plan for Exploratory Objectives	80
10.7.	Sample Size	80
10.7.1.	Accrual Estimates.....	80
10.7.2.	Sample Size Justification.....	80
10.7.3.	Effect Size Justification	81
10.8.	Criteria for Future Studies	81
10.9.	Descriptive Statistics and Exploratory Data Analysis	81
11.	REFERENCES.....	82
12.	PROTOCOL HISTORY	86
13.	APPENDICES.....	90
13.1.	Appendix A. Revised RECIST guideline (Version 1.1).....	90
13.2.	Appendix B. EORTC Quality of Life Questionnaires	91

PROTOCOL SYNOPSIS

Study Title	A Phase IIa Study Assessing QBS72S For Treating Brain Metastases of Breast Cancer
Study Drug	QBS72S
Study Description	A Phase IIa, single-group, cytotoxic chemotherapy treatment trial aimed at assessing efficacy and safety of novel agent QBS72S, which targets LAT1. All participants will receive study drug once monthly until disease progression. Enrolls participants diagnosed with parenchymal or leptomeningeal brain metastases of breast cancer.
Study Phase	Phase IIa
Study Purpose	Treatment
Primary Objective	Test of the preliminary efficacy of the intracranial anti-tumor activity of QBS72S through overall response rate (ORR) in Cohort 1 (Stages 1+2).
Primary Endpoint	ORR will be measured using modified Response Assessment in Neuro-oncology for Brain Metastases (mRANO-BM) and RECIST 1.1 response criteria. ORR will be measured by response rate across primary objective evaluable participants in Cohort 1 (Stages 1+2). A successful response is defined as $\geq 30\%$ decrease in sum total diameter of all target lesions compared with baseline across 2 or more sets of scans, provided that steroid dose is lower or the same as baseline and neurological evaluations are stable or improved.
Secondary Objectives	• Test of the preliminary efficacy of the intracranial anti-tumor activity of QBS72S through progression-free survival (PFS) in Cohort 1 Stage 1 and 2. • Test of the preliminary efficacy of the intracranial anti-tumor activity of QBS72S through overall survival (OS) in Cohort 1 Stage 1 and 2. • Test of the preliminary efficacy of the intracranial anti-tumor activity of QBS72S through durations of response (DoRs) in Cohort 1 Stage 1 and 2. • Evaluate safety of QBS72S treatment in Cohort 1 (Stages 1+2), Cohort 2, and Cohort 3.
Secondary Endpoints	• PFS is defined as the length of time that participants do not experience confirmed disease progression measured from the date of diagnosis of their study disease to the date of confirmed disease progression. • OS is defined as the length of time that participants survive from the date of diagnosis of their study disease to the date of their death. • DoR is defined as the length of time participants do not experience confirmed disease progression. Two metrics will be used, DoR_{prog} will be measured from the date of first IP to the date of confirmed disease progression, and DoR_{DoD} will be measured from the date of first IP to the date of death. • Safety is defined as a listing of all adverse events, graded according to CTCAE v5.0.

Exploratory Objectives

- Evaluate the intra-cranial progression free survival, overall survival, and durations of response in Cohort 2 and Cohort 3.
- Evaluate the overall response rate, progression free survival, overall survival, and durations of response in systemic disease sites in participants treated with QBS72S in Cohort 1 (Stages 1+2), in Cohort 2, and in Cohort 3.
- Determine pharmacokinetics in CSF of QBS72S in Cohort 2 and Cohort 3.
- Explore potential cellular and gene expression biomarkers of disease and therapeutic response in Cohort 1 (Stages 1+2), Cohort 2, and Cohort 3.
- Explore relationship between LAT1 expression and response to treatment via IHC analysis of primary tumor in Cohort 1 (Stages 1+2), Cohort 2, and Cohort 3.

Exploratory Endpoints

- PFS, OR, and DoR will be measured in Cohort 2 and Cohort 3 participants using the definitions laid out in the primary and secondary endpoints.
- ORR, PFS, OR, and DoR will be measured in the sub-set of participants with evaluable systemic disease sites.
- Pharmacokinetics will be measured by concentrations of QBS72S in participant CSF.
- Biomarkers will be examined through extracting and analyzing freely circulating cells within the CSF. Gene expression and cytological analyses of the CSF, brain tumor tissue, and primary tumor tissue will be done to identify potential biomarkers of disease for future exploration.
- LAT1 expression profile of the primary tumor will be determined through IHC staining. Response to treatment refers to the ORR, PFS, OR, and DoR calculated in the primary and secondary endpoints.

Indication

Participants diagnosed with breast cancer who have developed brain metastases.

Sample Size

We expect to accrue a total of 45 evaluable participants across all cohorts. We expect to accrue 15 evaluable participants for Cohort 1 Stage 1 and an additional 10 evaluable participants for Cohort 1 Stage 2 for a total of n=25 across the entirety of Cohort 1. We expect to accrue 10 evaluable participants in Cohort 2. We expect to accrue 10 evaluable participants in Cohort 3. See Section 10.7 Sample Size.

Eligibility Criteria

Eligible participants will be adult cancer patients with a Karnofsky Performance Status (KPS) ≥ 60 , diagnosed with primary breast cancer that has metastasized to the brain. Eligibility criteria are extensive. See Eligibility Criteria and Participant Eligibility Checklist at Section 3.1 and discussion at Section 6.2.2.1.

Dose

18 mg/m² and 12 mg/m²

**Treatment and
Procedural Summary**

All participants will receive IV injection of 18 mg/m² QBS72S once monthly until disease progression. This study will monitor lab values, physical exams, KPS, vitals, quality of life, medications, and AEs, and will collect imaging data. Cohort 2 and Cohort 3 participants may have Ommaya reservoirs placed and CSF sampled from these reservoirs throughout the study. Dose may be reduced to 12 mg/m² if participant is experiencing intolerable toxicity or infusion reaction despite supportive care, but there is evidence of potential clinical benefit to continuing.

**Statistical
Considerations**

The study will employ Simon's two stage design [24], which is one of the most common multi-stage designs used in Phase 2a clinical trials that allows flexibility regarding the null and alternative hypotheses while also allowing stopping for futility. The null hypothesis that the true response rate is 10% will be tested against a one-sided alternative. See Section 10.2.1.1 for the definition of primary objective evaluable participants. In the first stage, 15 primary objective evaluable participants will be accrued and treated with QBS72S. If there are one or fewer responses in these 15 participants, the study will be stopped. Otherwise, 10 additional evaluable participants will be accrued for a total of n=25. The null hypothesis will be rejected if six or more responses are observed in these 25 participants. This design yields a type I error rate of < 5% and power of 80% when the true response rate is 30% (see Table 5).

Study Design Considerations to Ensure Adequate Power

n	n1	R1	R2	Type 1 Error	Power	EN ₀	Probability of early stopping
25	15	1	5	0.0328	0.8017	19.5	0.5490

n is the total number of subjects

n₁ is the number of subjects accrued during stage 1

r₁, if **r₁** or fewer responses are observed during stage 1, the trial is stopped early for futility

r₂, if **r₂** or fewer responses are observed by the end of stage two, then no further investigation of the drug is warranted

EN₀ is the expected sample size for the trial when response rate is **p₀**

All participants regardless of LAT1 expression profile will continue to receive treatment every four weeks until confirmed disease progression, unacceptable toxicity, withdrawal from the study, or the clinician's decision to conclude treatment. The study will track participant clinical outcomes until death.

Study Duration

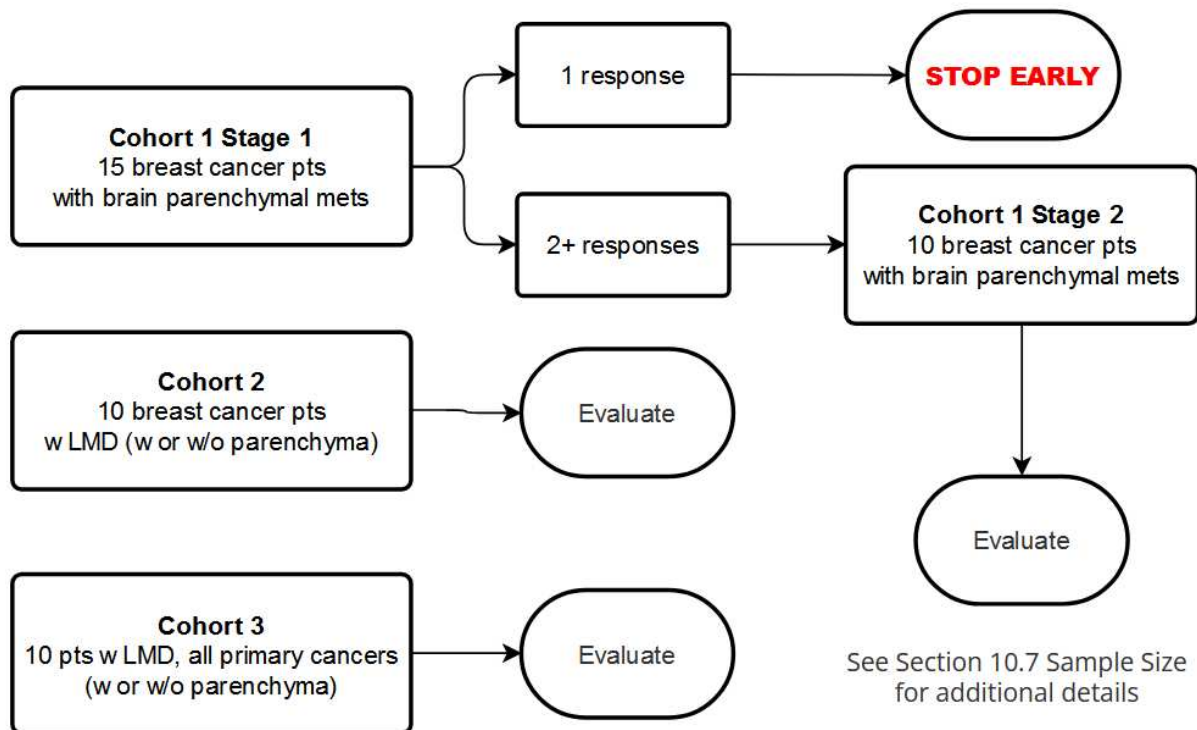
The study will reach primary completion when the last of the ORR study data, including all follow up, is expected to be collected, i.e., all enrolled participants have either withdrawn or received six cycles of QBS72S. The study will likely reach primary completion around 30 months from the time the study opens to accrual. Accrual will likely take a total of 26 months, the total treatment period will likely span a total of 28 months, and the total follow-up period will span from Month 2 to Month 30, for a total of 28 months span.

The study will reach total completion when the last study data, including all follow-up data, has been collected, and analysis is complete. This is expected to occur when all participant data has been analyzed per Section 10 Statistical Considerations. The study will likely reach study completion around 48 months from the time the study opens to accrual. The study opened in August 2022. The anticipated primary completion date is in February 2025. The anticipated study completion date is in August 2026.

Participant Duration

A participant who enrolls will have up to 28 days in the screening phase before treatment begins. Treatment is offered on an ongoing basis, and cessation of treatment is based on disease progression. We expect participants to complete 2 to 5 month-long cycles of treatment on average. Once participants have completed treatment, they will undergo long term follow up until withdrawal or death.

PROTOCOL SCHEMA



LIST OF ABBREVIATIONS & TERMS

Accrual date	Participant's on-study date (per OnCore)	LAT1 or 2	Large amino transporter type 1 or 2
AE	Adverse event	LC ₅₀	Lethal concentration 50%
aPTT	Activated partial thromboplastin time	LMD	Leptomeningeal
BC	Breast cancer	LMD	Leptomeningeal disease
BLI	Bioluminescence imaging	min	Minute
cm	Centimeters	mg	Milligrams
CL	Clearance	mm	Millimeters
CMC	Chemistry, manufacturing, and controls	mmHg	Millimeters of mercury
CNS	Central nervous system	MRI	Magnetic resonance imaging
CRF	Case report form	MTD	Maximum tolerated dose
CSF	Cerebrospinal fluid	NADPH	Nicotinamide adenine dinucleotide phosphate
CTCAE	Common terminology criteria for adverse events	NSCLC	Non-small cell lung cancer
CYPs	Cytochrome P450s	ORR	Overall response rate
d	Day	On-study date	Defined (per OnCore) as date of participant's first research-related procedure, scan, or treatment
DCE	Dynamic contrast enhancement	PARP	Poly (ADP-ribose) polymerase
DNA	Deoxyribose nucleic acid	PD	Protocol director
DSC	Dynamic susceptibility contrast	PD-1	Programmed cell death protein 1
DSMC	Data safety monitoring committee	PD-L1	Programmed cell death ligand 1
ECG	Electrocardiogram	PI	Principal investigator
EIAEDs	Enzyme-inducing antiepileptic drugs	PK	Pharmacokinetic
EOI	End of infusion	PLT	Platelet
FDA	Food and drug administration	PO	By mouth
GBM	Glioblastoma	q	Approximately
GCP	Good clinical practice	RBC	Red blood cell
Gd-MRI	MRI with gadolinium contrast agent onboard	RET	Reticulocyte
GLP	Good laboratory practice	SAE	Serious adverse event
Gy	Gray, radiation unit measurement	SCI	Stanford cancer institute
HNSTD	Highest non-severely toxic dose	SOP	Standard operating procedure
IC ₅₀	Inhibitory concentration 50%	SRC	Scientific review committee
IND	Investigational new drug	STD ₁₀	Severely toxic dose in 10%
INR	International normalized ratio	TK	Toxicokinetic
IRB	Institutional review board	TNBC	Triple-negative breast cancer
IV	Intravenous	US	United States
kg	Kilograms	Vd	Volume of distribution
KPS	Karnofsky performance status	w	Week
LAR	Legally authorized representative	WBC	White blood cell

1. INTRODUCTION

1.1. Study Rationale

Brain tumors can develop either directly from brain cells themselves (as in the case of glioblastomas) [1] or from cells that metastasize to the brain from peripheral tumors (e.g., breast or lung tumors) [2]. Metastatic brain tumors (also called “brain metastases” or “brain mets”) are by far the more common type of brain tumor, with an estimated 200,000 to 300,000 new cases diagnosed each year [3]. Brain metastases are a particularly challenging cancer sub-type to treat because the blood brain barrier blocks entry for the vast majority of systemic chemotherapeutic agents approved for use today.

Breast cancer is the second most common cause of cancer death among women, and the most common source of brain metastases. These women are faced with only 6 to 9 months survival from diagnosis of brain metastasis and a significant decrease in quality of life due to the tumors themselves and the surgery and radiation used to treat them [7, 8]. The chemotherapeutic options to treat brain metastases are at this time also very limited, with only Enhertu providing a relatively high amount of brain penetrance [54], which is only indicated for people with HER2 positive disease [53].

Grades 3 and 4 breast cancer lesions express high levels of L-type amino acid transporter 1 (LAT1). The TNBC sub-type expresses the highest levels among the other hormone expression sub-types [50], making it a poor candidate for hormone-based systemic chemotherapy but a good target for LAT1-targeted therapy.

A sub-set of cancer patients with brain metastases will go on to develop leptomeningeal disease (LMD), where cancer cells metastasize to a thin, diffuse coating across the meninges of the brain. Sometimes referred to as a sugar-coating for its appearance on scans, LMD's diffuse nature makes it infeasible to treat with targeted radiotherapy or surgery, and there are currently no FDA-approved drugs to treat LMD [31]. Recent investigation has shown that patients with LMD face even shorter survival times of 2.5 months and a significant decrease in quality of life compared to patients with tumors of the brain parenchyma [32-35].

The initial pre-clinical data supporting this study focused on breast cancer, specifically on TNBC. However, since the opening of this study, new data have been published to suggest that all cancer types metastatic to the brain correlate with high LAT1 levels – as many as 98% of all brain metastases have moderate to high levels of LAT1 expression [49]. Given that patients with LMD face poor survival time and severe quality of life limitations as previously discussed, we propose to open a third arm of the study that enrolls participants with LMD, regardless of primary cancer type. We expect most of the enrollments to this third arm of the study to be patients with primary lung cancer, as lung and breast make up over half of all primary sites of brain cancer metastases [56].

Our aim is to address a two-fold need with this study: the need for an additional brain-penetrant chemotherapeutic agent that is able to treat all sub-types of brain metastases, and the need for a non-hormone based chemotherapeutic agent able to treat for treatment-resistant TNBC.

1.2. Background

1.2.1. Overview of Study Agent

Quadriga's technology is based on the discovery that many aggressive cancers over-express the L-type amino acid transporter 1 (LAT1) as a means of securing the amino acids that cancer cells need to support their growth and metabolic needs [2, 15, 16]. High LAT1 expression is correlated with poor survival outcomes in virtually all cancer types investigated to date, including breast, brain, liver, pancreatic cancers. Of particular relevance to brain cancers, LAT1 is also normally expressed on the endothelial cells that form the blood brain barrier, where it facilitates the transport of amino acids as nutrients to the brain. Certain amino acid-based drugs, such as L-Dopa for Parkinson's disease and gabapentin for seizures are known to be transported across the blood brain barrier by LAT1.

Quadriga has exploited these findings to develop novel amino acid mimetics that are transported by LAT1 and kill cancer cells. Because these drugs are transported by LAT1, they can both cross the blood brain barrier and be actively transported into cancer cells, where they cause DNA damage and then the kill cancer cells. Because these drugs are transported much more effectively by LAT1 than by other cellular transporters, they selectively enter tumor cells within the brain and not surrounding normal cells.

Should QBS72S prove efficacious in treating breast cancer patients with brain metastases, it will hold promise for treating patients with other cancers that develop brain metastases, including lung cancer and melanoma. Moreover, since LAT1 is highly expressed on many forms of aggressive cancer as well as brain metastases [49], QBS72S may be suited to treating both systemic disease and CNS metastases, particularly in settings such as breast cancer with leptomeningeal metastases where there are few therapeutic choices.

There is a very large unmet need among patients with brain metastases. We believe that QBS72S will have favorable impact on disease progression and may ultimately be used in combination with other drugs such as PD-L1 inhibitors to achieve more durable responses. Quadriga intends to develop QBS72S to treat brain cancers, both primary (such as glioblastoma) and secondary (such as breast cancer brain metastases). This study will provide the initial data on efficacy in brain mets and will lay the groundwork for potentially treating the 200,000 to 300,000 patients per year in the US who develop brain metastases from breast, lung, and other cancers [3].

1.2.2. Mechanism of Action for QBS72S

QBS72S belongs to a type of alkylating agent that is employed in cancer chemotherapy associated with extensive and durable DNA damage utilizing LAT1 as both a transport and targeting mechanism that is currently being investigated in participants with relapsed refractory breast cancer brain metastases.

Quadriga has developed a novel amino acid analogue integrated with a DNA alkylating moiety, QBS72S, that leverages the blood-brain-barrier-specific large amino transporter type 1 (LAT1) for active transport into the brain. LAT1 is expressed in upwards of 98% of brain metastases

and is highly expressed in TNBC metastases [49], but is not expressed in normal brain cells, enabling selective anti-tumor activity by QBS72S. Our preclinical studies have shown that QBS72S selectively kills TNBC cells in vitro and effectively decreases tumor growth and prolongs survival in vivo in an aggressive human TNBC brain metastasis mouse model.

Based on our pre-clinical studies, we expect that QBS72S will cross the blood-brain barrier at effective concentrations, be actively transported into the cancer cells, and exhibit selective cytotoxicity towards cancer vs normal cells. Moreover, we anticipate that QBS72S will be active against systemic breast cancer disease in addition to the brain mets.

1.2.3. Non-clinical Background

1.2.3.1. QBS72S is Selectively Taken up by and Toxic to LAT1-expressing Cells in vitro

In a set of in vitro experiments, we induced LL-PKI cells to express either LAT1 or LAT2 and assessed the uptake and toxicity of QBS72S in each population. QBS72S showed a 50-fold preference for LAT1, with an IC_{50} of 21 μ M for LAT1 versus 1100 μ M for LAT2. QBS72S was also 4 to 8 times more potent in killing high LAT1-expressing cells compared to uninduced cells (Figure 1). These findings suggested that LAT1 selectivity conferred tumor selectivity for QBS72S, and this selectivity could reduce the potential for unintended off-target effects at therapeutic doses.

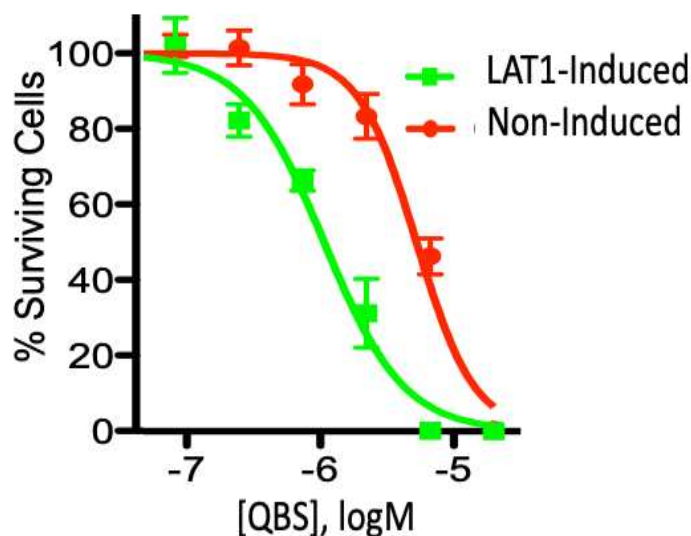


Figure 1. QBS72S is 4- to 8- times more toxic in cells overexpressing LAT1 compared to non-induced cells. QBS72S was preferentially toxic to high LAT1-expressing LL-PKI cells in vitro. LAT1 inducible LL-PKI cells were treated with various concentrations of QBS72S and surviving cells were quantified 10 days after treatment. Data are expressed as the mean $\pm$ SEM relative to untreated cells.

1.2.3.2. QBS72S Exerts its Cytotoxic Activity by Promoting Double-strand Breaks in DNA

Treatment of GBM cells in vitro with QBS72S caused a dose-dependent increase in the phosphorylation of H2AX, a DNA damage repair protein, at Ser 139 (data not shown). This

confirms that QBS72S's mechanism of cytotoxicity is the formation of double-strand breaks after DNA damage [17].

1.2.3.3. **QBS72S is Less Toxic than Melphalan in Normal Cells in vitro**

In in vitro experiments with normal human astrocytes, mouse fibroblasts, and bone marrow progenitor cells, QBS72S was less toxic than melphalan, another amino acid-based N-mustard (Table 1). This further strengthened our expectation that QBS72S may have fewer unintended off-target effects compared to other N-mustards.

Table 1. *In vitro* Cytotoxicity of QBS72S Compared to Melphalan

Compound	LC ₅₀ (μM)			
	Normal Human Astrocytes	Mouse Fibroblasts (3T3)	Bone Marrow Progenitors	
			Myeloid	Erythroid
QBS72S	19	9	2.2	2.1
Melphalan	0.9	2	1.4	0.42

1.2.3.4. **QBS72S Slows the Growth of TNBC and Other Tumor Cell Types in vitro and in Flank Xenografts**

QBS72S inhibited growth and induced apoptosis in cell lines from numerous tumor types, including TNBC tumors (MDA-MB-231 cells, Figure 2), glioblastoma, melanoma, leukemia, lymphoma, prostate, colon, and lung. The drug's cytotoxicity was dose-dependent, with LD₅₀ values ranging from 1.4 to 20 μM. To test the efficacy of QBS72S *in vivo*, MDA-MB-231 TNBC cells were implanted into the flanks of nude mice, and the tumors were allowed to grow to ~200 mm³. Mice were then treated with QBS72S or vehicle by IV once a week for 8 weeks. Tumor volume was measured 2 to 3 times per week. At doses of 5 mg/kg, QBS72S significantly inhibited TNBC tumor growth when administered either 1 or 3 times per week (Figure 3). QBS72S also suppressed the growth of other tumor types in xenografts (i.e., melanoma, prostate, acute myeloid leukemia, & myeloma) in a dose dependent manner (data not shown). These findings confirmed QBS72S exhibits anti-tumor activity *in vivo*.

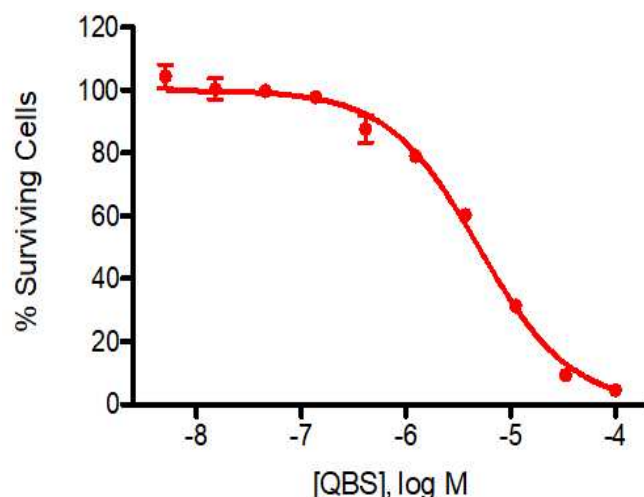


Figure 2. QBS72S showed dose-dependent cytotoxicity against MDA-MB-231 cells in vitro.

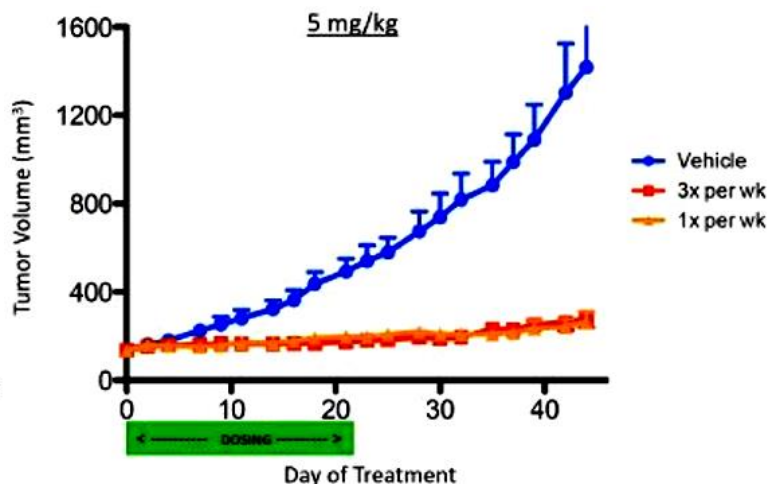


Figure 3. QBS72S significantly inhibited growth of MDA-MB-231 cells implanted in the flanks of nude mice.

1.2.3.5. QBS72S Crosses the Blood Brain Barrier and Accumulates in Tumor Tissues but not Normal Tissue

Using [^{14}C]-QBS72S, we conducted quantitative whole-body autoradiography studies in tumor-bearing animals to quantify blood brain barrier penetration and brain tumor accumulation. One hour after 5 mg/kg of [^{14}C]-QBS72S was dosed IV into a mouse bearing an intracranial glioblastoma tumor (LN229), the tissue concentration of radioactivity was essentially equivalent in blood and tumor but significantly lower in surrounding normal tissue (Table 2), indicating QBS72S crosses the blood brain barrier and preferentially accumulates in tumor. These findings confirmed the LAT1-selective transport and accumulation of QBS72S in vivo.

Table 2. Accumulation of QBS72S in Tissues over Time

Tissue	Tissue Concentration of QBS72S ($\mu\text{g equiv / g tissue}$)		
	1 hour	8 hour	24 hour
Blood	2.20	0.58	0.73
Brain	0.42	0.10	0.11
Tumor	2.01	0.39	0.33

1.2.3.6. QBS72S is Effective Against Intracranial Tumors

We found that QBS72S slows tumor growth and improves survival in two different mouse models of intracranial tumors: TBNC brain mets and glioblastoma.

Intracranial GBM model – QBS72S's effect on the growth of intracranial GBM tumors was studied using an orthotopic intracranial xenograft model transplanted with two different cell lines, U251 and LN229, both modified with luciferase. Both cell lines express LAT1 and form tumors

when injected intracranially into the left frontal cortex of the brain of nude mice. This is a well-characterized, robust model that is sensitive to TMZ, which we used as a control. The tested doses ranged from 2 to 15 mg/kg. In all studies, 8 to 10 mg/kg dosed intravenously once a week for 3 to 6 weeks showed good tolerability and demonstrated reproducible and robust tumor suppression with increased survival. Tumor growth, when measured on the last day of survival for all control animals, was suppressed by 96% in the U251 model (Figure 4) and 87% in the LN229 model. In the U251 model, the median survival time increased from 31 to 37 days in vehicle-treated mice to 56 to 70 days in QBS72S-treated mice. In the LN229 model, the median survival time increased from 53 to 56 to 68 to 74 days, respectively. Finally, when we combined radiation treatment (1.5 Gy for 5 days) with QBS72S in the U251 model, a significant benefit was seen in both tumor growth suppression and median survival times when compared to either monotherapy (i.e., either QBS72S or radiation treatment alone).

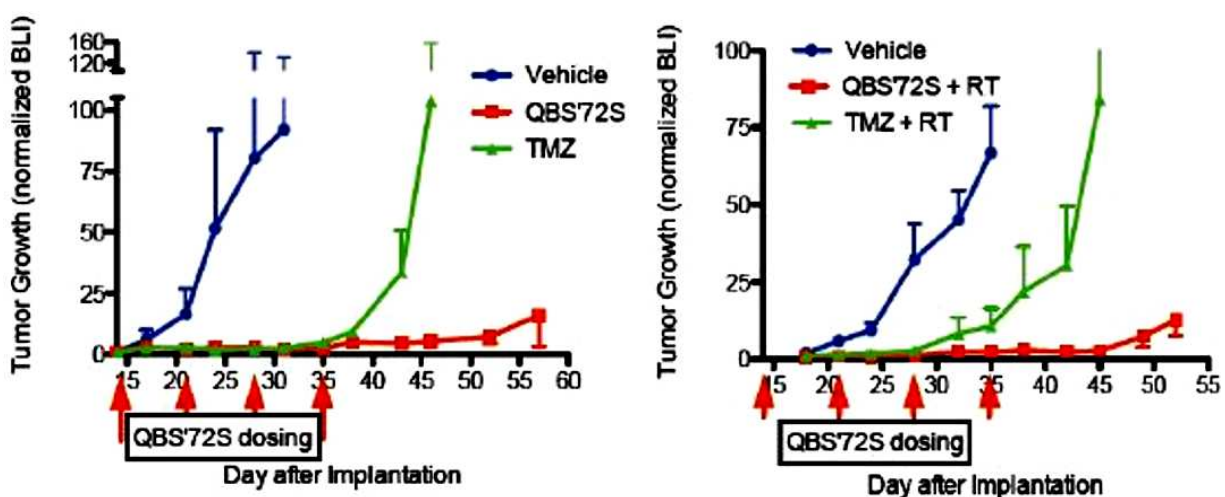


Figure 4. Intracranial U-251 (glioblastoma) xenograft model. 10 mg/kg QBS72S vs. 4 mg/kg temozolomide (TMZ). RT = radiation therapy

1.2.3.7. QBS72S in Breast Cancer Brain Metastases

Therapeutic breakthroughs for brain mets must be tested on cancer cells growing within the brain microniche. Dr. Melanie Hayden Gephart's lab at Stanford established a novel mouse model of TBNC leptomeningeal metastases (LMs) using a brain-tropic TNBC cell line, MDA-MB-231 BR3, expressing luciferase [18]. Injections of these cells into the internal carotid artery of NuNu mice produced brain mets in > 60% of mice, as determined by bioluminescence imaging (BLI), when more than 10,000 cells were injected. Once the BLI signal intensity from the metastatic spread reached 0.2 - 0.5 x 10⁶ photons/sec, mice were dosed IV with 8 mg/kg QBS72S once a week for 9 weeks. Tumor growth was monitored by BLI (Figure 5). Compared to vehicle-treated controls, mice treated with QBS72S showed an increase in median survival from 25 to 52 days (Figure 6A; range 45-70d, $p < 0.002$). This prolonged survival was accompanied by a significant (94%) decrease in tumor growth (Figure 6B; $p < 0.0001$). QBS72S was well-tolerated with no adverse effect on animal weight during the course of the study (Figure 6C). These findings show that in xenograft models, QBS72S is effective at suppressing the growth of both primary TNBC tumors and metastatic TNBC brain tumors.

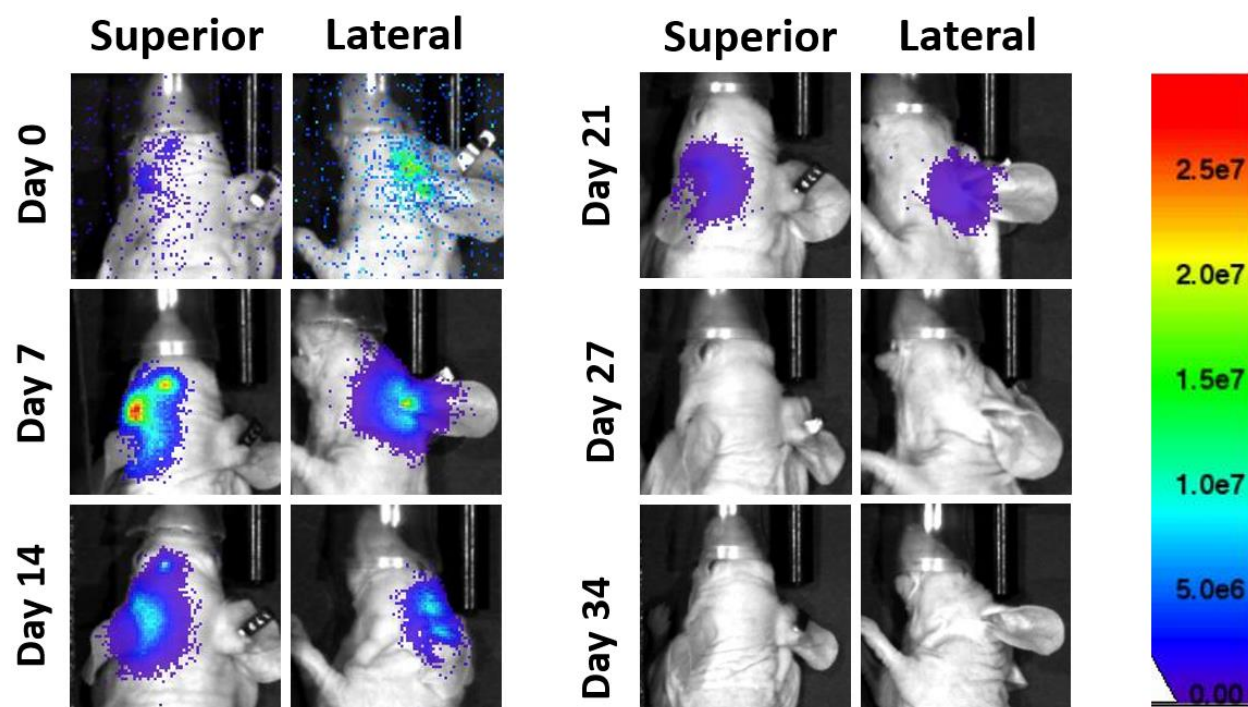


Figure 5. BLI of a QBS72S treated LM mouse. Tumors regressed completely in one of the LM mice treated with 8 mg/kg of the drug.

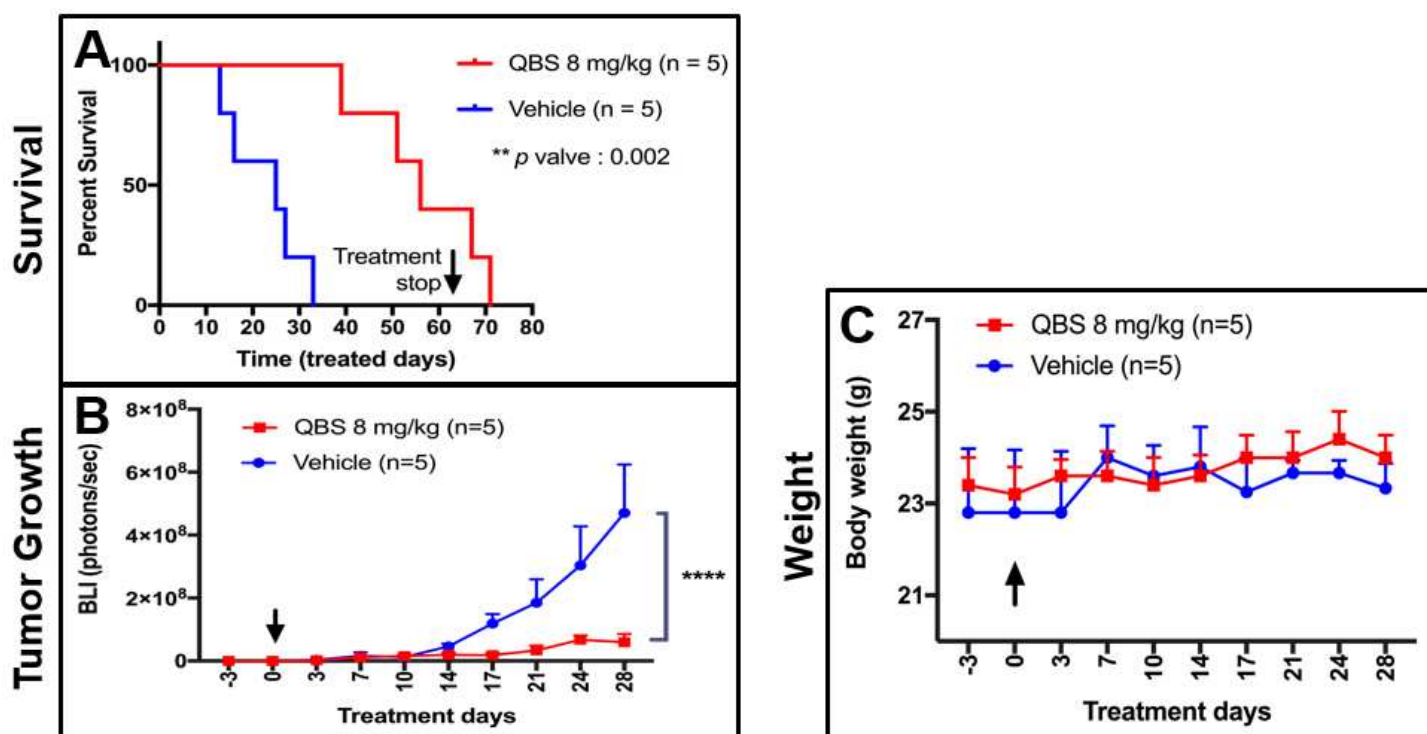


Figure 6. QBS72S decreases tumor growth and improves survival in the MDA-231-BR3-ICA LM disease mouse model. Mice with established TNBC LMD treated with QBS72S showed significant increase in survival (Figure 6A; Kaplan Meier curves) and decrease in tumor growth (Figure 6B; $p < 0.0001$) compared to vehicle control. QBS72S treatment was well tolerated with no adverse effect on animal weight (Figure 6C).

1.2.3.8. Non-clinical and Clinical Pharmacokinetics

We assessed the pharmacokinetic profile of QBS72S in several studies with male and female Sprague-Dawley rats and male and female Cynomolgus monkeys. In a single-dose dose proportionality study with IV administration of 0.5, 1.5, or 4.5 mg/kg QBS72S in rats, AUC_{0-t} demonstrated linear exposure across the dose range, and we did not observe any difference in volume of distribution (Vd) or clearance (CL) among the tested doses. In multiple repeated dosing (every 3 weeks or every 4 weeks) dose proportionality studies with IV administration of higher dose ranges (i.e., 2 to 12 mg/kg) in rats, we observed a modest difference by sex in AUC_{0-t} (< 2-fold higher for males) but no differences in C_{max} . As in the single-dose proportionality study, AUC_{0-t} demonstrated linear exposure across the dose range, and we did not observe any differences in Vd. Drug elimination (i.e., $t_{1/2}$ and CL) was modestly higher in males than females on days 1 and 29. Single- and repeated-dosing studies in monkeys also found that AUC_{0-t} demonstrated linear exposure across the dose range without differences in Vd or CL among the tested doses (i.e., 0.5 to 8 mg/kg). Importantly, we did not find any sex differences in AUC_{0-t} , $t_{1/2}$, CL, or any other PK parameter in monkeys.

1.2.3.9. Major Route of Elimination

The primary metabolism of QBS72S is spontaneous aqueous hydrolysis, typical of all bis-chloro alkylating agents. The half-life in athymic (nu+/nu+) xenograft mice was about 45 minutes. The hydrolysis products are biologically inactive. Based on bio-distribution studies with C^{14} labeled compound, the bulk of elimination occurs in the urine and feces [38, 39].

1.2.3.10. Non-Clinical Pharmacology and Toxicology Studies

Metabolism of QBS72S by liver microsomes of multiple species (human, monkey, dog, rat, mouse) were modest and equivalent (59.4, 62.0, 59.4, 59.4, and 62.6 $\mu\text{L}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}\text{protein}$, respectively), and the metabolism was NADPH independent, indicating non-enzymatic degradation. CYP inhibition assays with 0 to 100- μM QBS72S found that the IC_{50} is > 100- μM for CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4 (testosterone), or CYP3A4 (midazolam), indicating no clinically relevant inhibition.

We examined the potential for QBS72S to cause vascular and muscular irritation in New Zealand White rabbits, and we assessed hemolytic potential using red blood cells from the same species. In both cases, findings were negative, indicating QBS72S is unlikely to provoke vascular or muscular irritation or a hemolytic reaction.

We evaluated the TK profile of QBS72S in non-GLP and GLP studies in male and female Sprague-Dawley rats and male and female Cynomolgus monkeys. In a two-cycle (4-week interval between dosing) repeat dose toxicity study in male and female Sprague Dawley rats, QBS72S was administered (dosing on Day 1 and 29) via 3-minute IV infusion at 2.5, 5, and 10 mg/kg (male) or 12 mg/kg (female) followed by a 4-week recovery period. The high dose [10 mg/kg (male) or 12 mg/kg (female)] was not tolerated, as most animals in this group were found dead or moribund during Days 5 to 6. Males in the 5 mg/kg group and males/females in 10/12 mg/kg groups had discolored feces, watery feces, crissum staining, food consumption decrease, and/or body weight decrease. All QBS72S-treated animals showed white blood cell

(WBC) decrease and reticulocyte (RET) decrease within one week after each dosing, though a recovery trend was noted in the second week. All findings were recovered four weeks after treatment. Based on these findings, the severely toxic dose in 10% of animals (STD_{10}) is considered to be 5 mg/kg for rats.

A dose-escalating study established 5 mg/kg as the Maximum Tolerated Dose (MTD) of QBS72S in monkeys. Thus, we conducted a two-cycle repeat dose toxicity study with male and female monkeys dosed with vehicle or 1.25, 2.5, or 5 mg/kg of QBS72S on Day 1 and Day 29 followed by a four-week recovery period. The high dose group (5 mg/kg) exhibited decreased activity, nose hemorrhage, inability to rise, hindlimb swelling, hindlimb skin ulcer, and watery feces. All treatment groups showed decreases in WBC, red blood cell (RBC), platelet count (PLT), and RET with the two weeks after each dose, but a recovery trend was noted by the third week. All findings in the 1.25 and 2.5 mg/kg groups recovered four weeks after treatment but not in the 5 mg/kg group. Based on these findings, the highest non-severely toxic dose (HNSTD) in monkeys is 2.5 mg/kg.

Using doses up to the STD_{10} in rats and HNSTD in monkeys, we conducted single-dose stand-alone GLP safety pharmacology studies to assess the central nervous system (CNS), respiratory, and cardiovascular responses to QBS72S. We did not observe CNS, respiratory, or cardiovascular effects at any dose tested.

Based on these results, the adverse reactions to QBS72S observed in rats, rabbits, and monkeys are generally consistent with the published findings on other alkylating agents (e.g., melphalan) [19].

1.2.4. Clinical Background

The Phase 1 MTD study began mid-2020 at the St. George Private Hospital in Sydney in Australia and led by Professor Paul De Souza, a medical oncologist with over 20 years of experience. The local Ethics Committee/IRB has reviewed all documents pertaining to the study with minimal comments, and final approval is expected by the end 2021. The Phase 1 study in Australia does not require an open US IND.

One or more participants in the Phase 1 clinical trial studying QBS72S safety experienced the following problems at least once: anemia; balance problems; bleeding around the brain; chest infection; constipation; diarrhea; dizziness; fatigue; hallucination; hot flashes; insomnia; jaundice; loss of appetite; nausea; numbness, tingling, or pins-and-needles sensation in toes, fingers, arms, and/or legs; pain in joints, abdomen, arms, chest, or lower back; rash; shortness of breath; slow heart rate; swelling of lips; thrombocytopenia; urinary incontinence; and vomiting. There have been no acute hypersensitivity infusion reactions yet observed in people receiving QBS72S. In the similar drug melphalan, acute hypersensitivity reactions, including anaphylaxis, have occurred in approximately 2% of patients who received an intravenous formulation. Symptoms may include itchiness, redness swelling, and skin rashes and, in some people, dizziness, elevated heart rate, shortness of breath, and low blood pressure.

Most alkylating agents cause gastrointestinal side effects and dose limiting toxicity to bone marrow with the major effect being a decreased granulocyte count. In addition to the effect on

the bone marrow, alkylating agents are cytotoxic to mucosal cells resulting in oral mucosal ulceration and effects on the intestinal mucosa. Other organ systems affected by some alkylating agents include the lungs (pulmonary fibrosis) and the liver. The expected DLTs of QBS72S are bone marrow and gastrointestinal based on GLP toxicology studies. Cardiovascular, respiratory, or neurological toxicities had not been observed in GLP safety pharmacology studies of QBS72S. More information can be found in the Investigator's Brochure that accompanies this protocol.

The QBS72S program was discussed at a Quadriga (manufacturer) Cohort Review Committee on 15 and 16 May 2022. This meeting was a Data Safety Monitoring Board-like meeting. It was presented that 2 participants on the phase 1 MTD study experienced a DLTs. The first experienced a DLT of Grade 4 thrombocytopenia at dose level 24 mg/m². The dose in this phase 1 study was lowered from 24 mg/m² to 18 mg/m². One additional participant experienced a DLT at 18 mg/m² of Grade 3 anemia. The dose cohort was expanded from 3 participants to 6 participants; no additional DLTs were observed at this dose level. Based on these results, the Cohort Review Committee determined 18 mg/m² to be the highest safe dose level in human participants. This dose (18 mg/m²) has been chosen for the current Quadriga Phase II trial as the therapeutic dose and is the dose to be used in this study, IRB-63041.

1.2.5. Importance of the Clinical Trial

Currently there are no FDA-approved drugs available to specifically treat brain metastasis in breast cancer patients, especially in TNBC. Chemotherapies such as taxanes and anthracyclines have been used to slow systemic disease but these do not cross the blood-brain barrier. More recently, the PD-1 inhibitor atezolizumab, in combination with nab paclitaxel, was approved to treat TNBC patients whose tumors are PD-L1 positive (depending on the specific measurement criteria, these patients represent approximately 20 to 55% of TNBCs) [1]. However, women with progressive, untreated brain metastases were excluded from the clinical trial on which the FDA approval was based and have largely been excluded from clinical trials in general: as of January 2020, we have found only 16 out of approximately 500 clinical trials for breast cancer that included patients with progressive, untreated brain metastases.

Brain metastases of breast cancer are currently treated by surgical resection, focused radiation therapy or whole brain irradiation. There are no other therapeutic options. Systemic chemotherapy that crosses the blood brain barrier is desperately needed. There are approximately 50,000 new cases of TNBC diagnosed per year in the US, and of those up to 50% will develop brain metastases. The prognosis for these patients is dire, with an average survival of only a few months. The brain metastases themselves, and/or the surgery +/- radiation used to treat them, result in patients experiencing exceedingly poor quality of life during their last few months.

There have been very few clinical trials of new drug candidates for the treatment of TNBC and only a handful for treating TNBC brain metastases. Currently used chemotherapies (generally taxanes and anthracyclines) have limited effect on systemic disease and do not cross the blood brain barrier.

A small pilot study with eribulin in participants who have previously not responded to anthracyclines and taxanes is being conducted in China [9]. Eribulin, like taxanes, is a microtubule disrupter. A study exploring the use of bevacizumab in TNBC patients with brain mets is just beginning [10]. Bevacizumab has shown no survival benefit when used as a steroid sparing agent in glioblastoma patients. In a parallel cohort, HER2+ breast cancer patients with brain mets will be treated with pyrotinib (an irreversible dual pan-ErbB receptor tyrosine kinase inhibitor) [11] which is not applicable to TNBC patients.

A Phase II study sponsored by Genentech [12] is currently recruiting TNBC patients with brain metastases to assess the potential for combining atezolizumab with stereotactic radiation. Atezolizumab is a PD-L1 checkpoint inhibitor and thus is useful in a subset of cancer patients whose tumors express PD-L1. The FDA has recently approved its use in metastatic TNBC patients, although the approval was based on a clinical trial that did not include patients with brain mets. Various reports indicate that about 20 to 55% of TNBC patients have PDL1-positive tumors are PD-L1 positive 1, and of those only 39% exhibited a response to atezolizumab [13]. Thus, a large number of TNBC patients are not candidates for checkpoint inhibitor therapy, and there is currently no evidence that such therapy will impact brain mets. Moreover, since checkpoint inhibitor therapy is not curative, additional agents are needed and could be used in combination in the appropriate patient subset.

There have been studies of lung cancer patients with brain metastases, but unfortunately very little success has been achieved to date. For example, veliparib (an inhibitor of the DNA damage response protein PARP) in combination with whole brain radiation therapy offered no improvement in clinical outcomes of non-small cell lung cancer (NSCLC) patients with brain metastases [14]. Significant efforts are underway in various pharmaceutical companies to develop more blood brain barrier permeable inhibitors of DNA damage repair pathways, but no results are yet available in either glioblastoma patients or patients with brain metastases of any tumor type.

In summary, there is a large unmet need for effective drugs to treat breast cancer patients with brain metastases, especially in TNBC, and few new compounds in development. We believe that QBS72S will be used in combination with surgery and/or radiation. We hope that other blood brain barrier permeable drugs will be developed that would be additive or synergistic with QBS72S.

1.3. Potential Risks and Benefits

1.3.1. Known Potential Risks of Study Drug QBS72S

Based on preliminary results from the Phase I trial, animal data, known effects of a component of this drug (melphalan), and initial safety data from this trial, we anticipate potential side effects to primarily represent bone marrow suppression (decreased neutrophil, white blood cell, lymphocyte, platelet, red blood cell counts). Additional potential side effects include cerebral edema, gastrointestinal toxicity (diarrhea, vomiting, mucositis), nausea, fatigue, and hypokalemia.

1.3.2. Known Potential Risks of Study Procedures

- Blood Draw/IV: Inserting a needle into the arm for drawing blood, or an IV catheter for infusion, can cause slight discomfort or bruising at the puncture site. Lightheadedness or fainting may occur. Rarely, an infection at the puncture site may occur.
- ECG: The adhesive pads used during this procedure may cause mild skin irritation or pull out some hairs when removed.
- MRI: There are no known health hazards from temporary exposure to the MR environment. However, the MR environment involves a strong, static magnetic field, a magnetic field that changes with time (pulsed gradient field), and radiofrequency energy, each of which carry specific safety concerns. First, the strong, static magnetic field will attract magnetic objects (from small items such as keys and cell phones, to large, heavy items such as oxygen tanks and floor buffers) and may cause damage to the scanner or injury to the participant or medical professionals if those objects become projectiles. Second, the magnetic fields that change with time create loud knocking noises which may harm hearing if adequate ear protection is not used. They may also cause peripheral muscle or nerve stimulation that may feel like a twitching sensation. The radiofrequency energy used during the MRI scan could lead to heating of the body. The potential for heating is greater during long MRI examinations.
- Personal Information: There is a risk of disclosure of personal health information (PHI).
- Ommaya Placement: The placement of a ventricular reservoir is within the standard of care for patients with leptomenigeal disease as these patients require frequent CSF sampling and occasionally the placement of intrathecal chemotherapy. The procedure carries an associated <1% risk of complication, including but not limited to bleeding, infection, need for reoperation, new or worsening neurologic deficit, complication of anesthesia, CSF leak, seizure, displacement or malfunction of the catheter, heart attack stroke, or venous thromboembolism.
- CSF Sampling: Each time the Ommaya reservoir is accessed there is a small chance of introducing infection. Removal of CSF can cause low pressure headaches or nausea. Mild pain is expected when the reservoir is accessed, however, this is generally much less than the placement of an IV.

1.3.3. Known Potential Benefits

There are no guaranteed benefits for taking part in this study. However, information obtained from participation in this study may benefit other people with cancer in the future and help determine if QBS72S is effective and safe.

2. STUDY DESIGN, OBJECTIVES, AND OUTCOMES

2.1. Study Design

2.1.1. Overall Design

The intervention model of this study is single group assignment. This study is a treatment trial. The study is open label. No masking is used. The study is non-randomized. The study will employ a Simon's two stage design.

2.1.2. Primary Purpose of the Study

The primary purpose of this study is treatment. This study is to evaluate the efficacy and safety of QBS72S in participants with advanced, relapsed, metastatic breast cancer with CNS involvement.

2.1.3. Study Cohorts

There are three treatment cohorts. Cohort 1 is comprised of two stages, Cohort 1 Stage 1 and Cohort 1 Stage 2. Cohort 2 is a single stage. Cohort 3 is a single stage.

In the first stage of Cohort 1 of this trial, 15 primary objective evaluable participants will be accrued to Cohort 1 Stage 1. For a definition of primary objective evaluable participants, refer to Section 10.2.1.1. If there are one or fewer responses in the 15 evaluable participants, the study will be stopped. A response is defined as a $\geq 30\%$ decrease in sum total diameter of all target lesions compared with baseline, sustained across two sets of scans taken approximately 4 weeks apart, steroid dose the same or lower compared with baseline scan, and stable or improved neurological examinations (see Section 10.2.1.2 Measurement Definition). Otherwise, 10 evaluable additional participants will be accrued for the second stage to form Cohort 1 Stage 2 for a total of 25 evaluable participants in Cohort 1.

Separately, the study will enroll up to 10 evaluable breast cancer participants with leptomeningeal disease (LMD) to Cohort 2 to assess the preliminary safety, tolerability, and blood brain barrier permeability of QBS72S. For a definition of exploratory objective evaluable participants, refer to Section 10.2.3.1.

As an add-on cohort, the study will enroll 10 evaluable participants with leptomeningeal disease (LMD) from any primary cancer to Cohort 3 to assess the preliminary safety, tolerability, and blood brain barrier permeability of QBS72S. For a definition of exploratory objective evaluable participants, refer to Section 10.2.3.1.

2.1.4. Scientific Justification for Study Design

The study's structure, which separates participants with parenchymal metastasis from participants with leptomeningeal disease, is necessary because these two etiologies behave very differently clinically. In particular, active leptomeningeal disease is often an exclusion criterion for clinical trials and has a much worse prognosis than active parenchymal metastases. However, these patients have limited treatment options and are excluded from most clinical

trials, so we have elected to include this population and examine the treatment paradigm's efficacy in this under-served population.

The study is open-label and not randomized in order to conduct a preliminary, smaller scale investigation of efficacy. Should this trial be successful, a randomized, blinded Phase III trial on a much larger scale will be considered.

This study uses historical controls of known growth rates in untreated brain metastases. The study design does not include actively enrolled participants receiving no IP for size and enrollment considerations.

2.1.5. Treatment Assignment

Treatment assignment is open label. No masking or blinding will be used. All participants will receive the same treatment with study agent QBS72S.

2.1.6. Justification for Dose

18 mg/m² is the recommended dose level per the unpublished results from the interim review committee of the Phase I study of the safety of QBS72S. Additionally, 12 mg/m² was tested and found by the interim review committee to be safe. The Phase I study is titled "A Phase 1, Open Label, Multi-Center, Single and Multiple Dose, Dose Escalation and Expansion Study to Assess the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of QBS10072S in Previously Treated Patients with Advanced or Metastatic Cancers with High LAT1 Signatures, and in Patients with Relapsed or Refractory Grade 4 Astrocytoma", NCT04430842, and is sponsored by Quadriga Australia Pty Ltd (Quadriga). This Phase I dose escalation study is being conducted at St. George Private Hospital and Sydney Southwest Private Hospital in New South Wales. Expected primary completion date is September 2022 and the expected study completion date is December 2022. This study has no masking and no randomization.

2.1.7. Study Timeline

The current Phase II study will likely reach primary completion around 30 months from the time the study opens to accrual. Accrual will likely take a total of 26 months, the total treatment period will likely span a total of 28 months, and the total follow-up period will span from month 2 to month 30, for a total of 28 months span.

The study will likely reach completion around 48 months from the time the study opens to accrual. The study opened in August 2022. The anticipated primary completion date is in February 2025. The anticipated study completion date is in August 2026.

2.1.8. End of Study Definition

This clinical trial is considered completed when participants are no longer being examined or the last participant's last study visit has occurred.

A participant is considered to have completed the study if they have completed all phases of the study including the last visit or the last scheduled procedure shown in Section 6.1 Schedule of Events.

2.2. Objectives

2.2.1. Primary Objective

Test of the preliminary efficacy of the intracranial anti-tumor activity of QBS72S through overall response rate (ORR) in Cohort 1 (Stages 1+2).

2.2.2. Secondary Objectives

- Test of the preliminary efficacy of the intracranial anti-tumor activity of QBS72S through progression free survival (PFS) in Cohort 1 (Stages 1+2).
- Test of the preliminary efficacy of the intracranial anti-tumor activity of QBS72S through overall survival (OS) in Cohort 1 (Stages 1+2).
- Test of the preliminary efficacy of the intracranial anti-tumor activity of QBS72S through durations of response (DoR) in Cohort 1 (Stages 1+2).
- Evaluate safety of QBS72S treatment in Cohort 1 (Stages 1+2), Cohort 2, and Cohort 3.

2.2.3. Exploratory Objectives

- Evaluate the intra-cranial progression free survival, overall survival, and durations of response in Cohort 2 and Cohort 3.
- Evaluate the overall response rate, progression free survival, overall survival, and durations of response in systemic disease sites in participants treated with QBS72S in Cohort 1 (Stages 1+2), in Cohort 2 and in Cohort 3.
- Determine pharmacokinetics in CSF of QBS72S in Cohort 2 and Cohort 3.
- Explore potential cellular and gene expression biomarkers of disease and therapeutic response in Cohort 1 (Stages 1+2), Cohort 2, and Cohort 3.
- Explore relationship between LAT1 expression and response to treatment via IHC analysis of primary tumor in Cohort 1 (Stages 1+2), Cohort 2, and Cohort 3.

2.3. ClinicalTrials.gov Registration, Outcomes, and Results

2.3.1. Data Sharing Statement

Pursuant to the International Committee of Medical Journal Editors (ICMJE), the following statement, regarding sharing of individual patient data (i.e., participant-level data) generated by interventional clinical trials, is provided.

It is not planned that individual participant data, including data dictionaries, will be made publicly available. Individual participant study data judged by the investigator to be pertinent to a participant's understanding of their medical condition and treatment options will be shared with that participant. Otherwise and as such, details of what, or when, or by what mechanism, data

and/or documents will be shared, who would share such data, or for what purposes or analyses, are not available or not applicable.

Although not individual participant data, pursuant to the requirements of [42CFR§11.48\(a\)\(5\)](#) (if applicable), the final IRB-approved protocol document with statistical analysis will be made available in the ClinicalTrials.gov results record.

2.3.2. Outcome Measures for ClinicalTrials.gov Results Reporting

2.3.2.1. Outcome 1 (Primary)

- **Title:** Overall Response against Intracranial Tumor Lesions, Cohort 1 (Stages 1+2)
- **Description:** The overall response against the target intracranial tumor lesions in Cohort 1 (Stages 1+2) participants was assessed by according to the modified Response Assessment in Neuro-oncology for Brain Metastases (mRANO-BM) and RECIST 1.1 response criteria. Clinical response means either a complete response (CR) or a partial response (CR). CR is defined as disappearance of tumor lesions, and PR is defined as $\geq 30\%$ reduction in diameter of tumor lesions. The outcome is reported the number of Cohort 1 (Stages 1+2) participants who achieved a CR or PR within 6 months from the start of treatment.
- **Time Frame:** 6 months
- **Completion of Primary Outcome:** The study is expected to reach Primary Completion in February of 2024.

2.3.2.2. Outcome 2 (Secondary)

- **Title:** Progression-free Survival (PFS), Cohort 1 (Stages 1+2)
- **Description:** Progression-free Survival (PFS) means to remain alive without tumor progression, assessed as a $\geq 25\%$ increase in tumor diameter. The outcome is reported as the number of participants in Cohort 1 (Stages 1+2) who remained alive at 2 months without tumor progression.
- **Time Frame:** 2 months

2.3.2.3. Outcome 3 (Secondary)

- **Title:** Overall Survival (OS), Cohort 1 (Stages 1+2)
- **Description:** Overall survival (OS) refers to remaining alive at the time of the assessment. The outcome is reported as the number of participants in Cohort 1 (Stages 1+2) who remained alive at 2 months.
- **Time Frame:** 2 months

2.3.2.4. Outcome 4 (Secondary)

- **Title:** Duration of Response (DoR), Cohort 1 (Stages 1+2)
- **Description:** Duration of Response (DoR) refers to length of time that a clinical response is maintained in Cohort 1 (Stages 1+2) participants. Clinical response means

either a complete response (CR) or a partial response (CR). CR is defined as disappearance of tumor lesions, and PR is defined as $\geq 30\%$ reduction in diameter of tumor lesions. The outcome was assessed at 6 months and is reported as median DoR with standard deviation for Cohort 1 (Stages 1+2) participants who achieved a clinical response.

- **Time Frame:** 6 months

2.3.2.5. Outcome 5 (Secondary)

- **Title:** Related Adverse Events (AEs)
- **Description:** Toxicity refers to drug-related adverse events (AEs). The outcome is reported as the number of related AEs that were serious per 21CFR§312.32 or non-serious, and by severity (mild, moderate, severe, life-threatening, or fatal), numbers without dispersion. Life-threatening events will only be those that are clinically life-threatening, and not merely a laboratory value without significant clinical symptoms.
- **Time Frame:** 30 days following the last administration of study drug.

3. PARTICIPANT SELECTION

3.1. Eligibility Criteria and Participant Eligibility Checklist

Inclusion and Exclusion Criteria are provided on the Eligibility Checklist which may be extracted from this document for use in screening potential participants.

The Participant Eligibility Checklist must be completed in its entirety for each participant prior to receiving the first infusion. The completed, signed, and dated checklist is retained in the participant's electronic medical record.

Participant Eligibility Checklist

I. Protocol Information

Protocol Title:	A Phase IIa Study Assessing QBS72S For the Treating Brain Metastasis of Breast Cancer
OnCore number:	BRN0051
Principal Investigator:	Dr. Melanie Hayden Gephart

II. Participant Information

Participant Name / Unique ID: /		
Gender	☐ Male	☐ Female

III. Eligibility Criteria

3.1.1. Inclusion Criteria

Prospective Participant Must Meet ALL these Inclusion Criteria to be Eligible	Yes	No	Supporting Documentation *
1. The participant must be 18 years or older.	☐	☐	
2. The participant must have a Karnofsky Performance Status (KPS) of 60 or above.	☐	☐	
3. One of the following: a. Cohorts 1 and 2: The participant must have a histologically-confirmed breast cancer primary tumor, either confirmed via primary specimen or via metastasis site, having developed brain metastases (parenchymal or LMD) after a prior cytotoxic chemotherapy regimen. b. Cohort 3: The participant must have a histologically cancerous primary tumor, either confirmed via primary specimen or via metastasis site, having developed brain metastases (parenchymal or LMD) after a prior cytotoxic chemotherapy regimen. There is no restriction on prior cycles of systemic therapy for any primary cancer type. Breast cancer is not excluded from Cohort 3.	Cohort 1 ☐ Cohort 2 ☐ Cohort 3 ☐	Cohort 1 ☐ Cohort 2 ☐ Cohort 3 ☐	

Prospective Participant Must Meet ALL these Inclusion Criteria to be Eligible	Yes	No	Supporting Documentation *
4. One of the following: a. Cohort 1: A participant with breast cancer brain parenchymal tumors must have at least one non-irradiated tumor $\geq 3 \text{ mm}^2$ that can be seen on 2 or more separate acquired brain MRI sequences. b. Cohort 2: A participant with breast cancer primary and LMD must receive either: i. a brain MRI with contrast that supports the presence of LMD, or ii. a CSF cytology test that is either positive for or suspicious for malignancy. The presence of parenchymal brain metastases does not exclude these participants from Cohort 2. a. Cohort 3: A participant with LMD from any primary cancer site must receive either: i. a brain MRI with contrast that supports the presence of LMD, or ii. a CSF cytology test that is either positive for or suspicious for malignancy. The presence of parenchymal brain metastases does not exclude these participants from Cohort 3.	Cohort 1 ☐ Cohort 2 ☐ Cohort 3 ☐	Cohort 1 ☐ Cohort 2 ☐ Cohort 3 ☐	
5. For corticosteroids and anticonvulsants, the participant must either: c. not be taking any, or d. be taking stable or decreasing doses for ≥ 5 days prior to obtaining the screening Gd-MRI of the brain. The maximum allowable dose of corticosteroids is 8mg of dexamethasone per day, or the equivalent of another medication as assessed by a Neuro-Oncologist. No more than a 4mg per day increase of dexamethasone (compared to the previous day) is allowed for 7 days or fewer prior to C1D1, or the equivalent of another medication as assessed by a Neuro-Oncologist. Inhaled or topical steroids and adrenal replacement steroid doses are permitted at any dose.	☐	☐	

Prospective Participant Must Meet ALL these Inclusion Criteria to be Eligible	Yes	No	Supporting Documentation *
6. The participant must have completed washout from prior therapy before C1D1, as applicable: e. ≥ 7 days for Ommaya placement (Cohorts 2+3) and select kinase inhibitors¹ f. ≥ 14 days for select kinase inhibitors² and for small molecules and non cytotoxic systemic drugs not explicitly listed here g. ≥ 21 days for select kinase inhibitors³, and for monoclonal antibodies, cytotoxic chemotherapy, and checkpoint inhibitors not explicitly listed here h. ≥ 28 days for investigational drugs, radiotherapy, and major surgery. Minor procedures such as tumor biopsy are allowed with written approval from the PI All significant toxicities from previous anticancer therapy must have stabilized to a new baseline or have resolved. Participation in long term follow up in another clinical trial is allowed if no procedures will be performed which may interfere with the interpretation of study results. ¹Kinase inhibitors with ≥ 7-day washout: erlotinib, osimertinib, neratinib, cetuximab, gefitinib, panitumumab, dacomitinib, lapatinib, necitumumab, mobocertinib, vandetanib, crizotinib, ceritinib, alectinib, brigatinib, lorlatinib, entrectinib, larotrectinib, selipercatinib, pralsetinib, lenvatinib, neratinib, trastuzumab, dacomitib, lapatinib, pertuzumab, margetuximab, vemurafenib, dabrafenib, encorafenib, and binimetinib. ²Kinase inhibitors with ≥ 14-day washout: tucatinib, trametinib, niraparib, rucaparib, olaparib, talazoparib, and veliparib. ³Kinase inhibitors with ≥ 21-day washout: vandetanib, cabozantinib, fam-trastuzumab deruxtecan, and ado-trastuzumab emtansine	☐	☐	

Prospective Participant Must Meet ALL these Inclusion Criteria to be Eligible	Yes	No	Supporting Documentation *
7. The participant must have adequate bone marrow function, including: i. $ANC \geq 1,500/mm^3$ or $\geq 1.5 \times 10^9/L$ j. $Platelets \geq 100,000/mm^3$ or $\geq 100 \times 10^9/L$ k. $Hemoglobin \geq 9 g/dL$	☐	☐	
8. The participant must have adequate renal function, including serum creatinine $\leq 1.5 \times ULN$ or estimated creatinine clearance $\geq 50 mL/min$. In equivocal cases, a 24-hour urine collection test can be used to estimate the creatinine clearance more accurately.	☐	☐	

Prospective Participant Must Meet ALL these Inclusion Criteria to be Eligible	Yes	No	Supporting Documentation *
9. The participant must have adequate liver function, including: l. Total serum bilirubin $\leq 1.5 \times$ ULN unless the participant has documented Gilbert syndrome who must have a total bilirubin < 3.0 mg/dl m. Aspartate and Alanine aminotransferase (AST and ALT) $\leq 2.5 \times$ ULN; $\leq 5.0 \times$ ULN if there is liver involvement by the tumor	☐	☐	
10. Any participant physiologically capable of becoming pregnant or getting a partner pregnant must agree to use highly effective contraception during study treatment and for 7 months after study discontinuation.	☐	☐	
11. Participants or their designated advocates must be willing to and capable of providing informed consent and willing and able to comply with all scheduled visits, treatment plan, laboratory tests, lifestyle considerations, and other study procedures.	☐	☐	

3.1.2. Exclusion Criteria

Prospective Participants Must <u>NOT</u> Meet ANY of These Exclusion Criteria	Yes	No	Supporting Documentation *
1. Participants with any other current, active malignancy unrelated to their primary cancer diagnosis, except for adequately treated basal cell or squamous cell skin cancer or carcinoma in situ.	☐	☐	
2. Participants with intolerance to or who have had a severe (Grade 3) allergic or anaphylactic reaction to any of the substances included in the investigational product: sulfobutylether- β -cyclodextrin, melphalan, bendamustine, chlorambucil or any nitrogen mustard chemotherapeutics.	☐	☐	
3. Participants who currently use or have an anticipated need for a contraindicated medication including live vaccines, natalizumab, nivolumab, ocrelizumab, palifermin, pimecrolimus, tacrolimus, tofacitinib, and EIAEDs, including phenytoin, phenobarbital, carbamazepine, fosphenytoin, primidone, and oxcarbazepine. The washout period for any live vaccine is 30 days prior to enrollment. There is no restriction for seasonal flu vaccines that do not contain live virus, nor any approved COVID vaccine.	☐	☐	
4. Participants who are pregnant or breastfeeding.	☐	☐	

Prospective Participants Must <u>NOT</u> Meet <u>ANY</u> of These Exclusion Criteria	Yes	No	Supporting Documentation *
5. Participants who have active medical or psychiatric conditions which, in the opinion of the Principal Investigator or a Sub-Investigator, would compromise or interfere with their ability to participate in the study.	☐	☐	

* All participant files must include supporting documentation to confirm participant eligibility.

V. Statement of Eligibility

By signing this form of this trial I verify that this participant is:

☐ Eligible / ☐ ineligible for participation in **Cohort 1** of the study

☐ Eligible / ☐ ineligible for participation in **Cohort 2** of the study

☐ Eligible / ☐ ineligible for participation in **Cohort 3** of the study

Study Coordinator printed name:	Date:
Signature:	
Investigator printed name:	Date:
Signature:	
Triple-check reviewer printed name:	Date:
Signature:	

3.1.3. Lifestyle Considerations

There are no specific restrictions during any parts of the study pertaining to lifestyle and/or diet (e.g., food and drink restrictions, timing of meals relative to dosing, intake of caffeine, alcohol, or tobacco, or limits on activity), or considerations for household contacts.

If a prohibited medication or procedure is indicated for care, an Investigator from the study team will discuss care options with the participant's care provider recommending the change. The participant may choose to withdraw from the study, or they may choose to stay on study and forgo the prohibited medication or procedure. For a list of prohibited medications see Section 5.7.6 Prohibited Concomitant Medications.

3.1.4. Screen Failures

Participants who consent to participate in the clinical trial, but who do not meet 1 or more Eligibility Criteria during the screening procedures are considered Screen Failures.

Screen Failures will not be considered enrolled participants, although they will be tracked on the Screening Log (with reason for ineligibility) and in the OnCore study management system.

Individuals who do not meet the criteria for participation in this trial, i.e., screen failure, because of out of window lab values may be rescreened. Potential participants who are re-screened will be assigned the same sequence number and OnCore participant identifier as for the initial screening.

3.2. Recruitment and Retention Procedures

3.2.1. Recruitment

Potential participants may be identified by the following methods:

- Review of the medical records of new or existing oncology patients for potential participants who meet eligibility criteria.
- Stanford physicians may refer patients to the study for screening. A study investigator and/or study coordinator will discuss the study with the participant during a patient visit or with a visit scheduled for the purpose of recruitment to the study. This study will be listed on the Stanford Clinical Trials website and ClinicalTrials.gov. Patients of a particular clinic, physician, or other caregiver who is NOT a study investigator will be contacted or approached only after their physician has previously notified the participant about the study and obtained approval for contact by the study team.
- Self-referral by participant after viewing study listing on ClinicalTrials.gov or the Stanford Clinical Trials website.
- External physicians may refer patients to the study for screening.

3.3. Informed Consent Process

All participants must be provided with a consent form describing the study with sufficient information for participants to make an informed decision regarding their participation. Study participants will provide written informed consent prior to the conduct of any study-specific procedures in accordance with institutional policies. The participant will be informed that they may withdraw from the study at any time and that their participation is entirely optional. The participant will receive a copy of the signed and dated consent document. The original signed copy of the consent document will be retained in the research file. An encounter note documenting the consenting process will be created in the hospital charting software EPIC detailing the process of consent and noting any special circumstances.

3.3.1. Non-English Speakers

If the participant's primary language is other than English, a short form consent document in the primary language of the participant will be provided. An interpreter fluent in the participant's primary language will be present throughout the consenting process and will translate. A witness will also be present throughout the consenting process – the translator may serve as the witness, or a family member of the participant. The witness is not required to speak the participant's primary language. The witness must not be the person obtaining consent. The person obtaining consent will fill out all information on the long form English consent form on behalf of the participant and will initial and date next to each mark made. The participant will sign the short form consent in their primary language and will be provided a copy of this short form consent form. The original short form consent form and the original long form English consent form will be retained in the study records. Copies of all documents will be uploaded to EPIC along with the encounter note documenting the consenting process.

3.3.2. Physical Limitations to Consent

The population of eligible participants includes persons who may exhibit physical limitations caused by their disease condition, including shakiness or weakness in one or both upper extremities. If the participant is fully capable of consent but unable to write legibly, the participant may make a mark on the consent form to indicate their consent. The person obtaining consent will write the date and the participant's name under their signature, and then will initial and date this addition. The person obtaining consent may also complete any questions asked on the consent form with the verbal answers given by the participant. The person obtaining consent will initial and date next to any questions they complete. The participant will receive a copy of the signed and dated consent document. The original signed copy of the consent document will be retained in the research file. An encounter note documenting the consenting process will be created in EPIC detailing the process of consent and noting any special circumstances, including the reason the participant was unable to write legibly, and a description of the participant's mark indicating consent.

3.3.3. Cognitive Limitations to Consent

The population of eligible participants includes persons who may exhibit limitations of understanding caused by their disease condition, including limitations to their capacity for fully informed consent. In this case, the person obtaining consent will identify the participant's Legally Authorized Representative (LAR) if necessary, requesting a copy of the documentation that gives the LAR authority to act on behalf of the participant. If acquired, this documentation will be uploaded to EPIC and a copy will be retained in the study records. The person obtaining consent will first verbally inform the participant that they will discuss the study and seek consent from their LAR. If the participant does not object, verbally or non-verbally, then the person obtaining consent will review consent with the LAR. The LAR will sign the consent form on behalf of the participant prior to any study procedures and after all of the LAR's questions could be asked and answered. The LAR will be informed that they may withdraw the participant from the study at any time and that the participant's participation is entirely optional. A copy of the signed consent form will be provided to the LAR, a copy will be uploaded to EPIC, and the original copy will be retained in the study records. An encounter note documenting the consenting process will be created in EPIC detailing the process of consent and noting any special circumstances, including full documentation of the LAR process. If the participant regains capacity to consent at any time during the course of the trial, the study team will immediately seek consent from the participant before any further study procedures occur.

3.4. Registration and Enrollment

3.4.1. Participant Registration Procedures

All participants who provide informed consent for this study will be registered in the Stanford OnCore Enterprise Research System ("OnCore") database regardless of the outcome of any eligibility screening.

At registration, the study coordinator will sequentially assign eligible participants a unique participant identification number.

3.4.2. Participant Enrollment Procedures

For this study, a participant is enrolled on the study when registered per Section 3.4.1 Participant Registration Procedures; has met eligibility per the screening procedures; and has been otherwise accepted into the study.

4. STUDY INTERVENTION

4.1. Investigational Drug

QBS72S for injection is supplied as a sterile white to off-white lyophilized powder in a single dose vial for intravenous use. Each vial contains 50 mg QBS72S free base and 2700 mg Betadex Sulfobutyl Ether Sodium, NF (see Table 3).

QBS72S is a cytotoxic drug. Follow institutional handling and disposal procedures for cytotoxic drugs.

Do not mix QBS72S with other injectable drug products.

Table 3: Composition of Drug Product

Intervention Name	QBS72S
Type	Drug
Dose Formulation	Lyophilized powder for solution for injection
Unit Dose Strength(s)	50 mg per vial
Dosage Level(s)	18 mg/m ² and 12 mg/m ²
Rout of Administration	Intravenous
Packaging and Labeling	Study Intervention will be provided in a vial. Each vial will be labeled with the investigational drug statement defined at 21CFR§312.6.

4.1.1. Name

The study agent is referred to in this protocol as QBS72S, also identified as QBS10072S. The chemical name is (S)-3-Amino-4-[5-[bis(2-chloroethyl)amino]-2-methyl-phenyl]butanoic acid. As of the writing of this protocol, there is not an established generic or trade name for this compound.

4.1.2. Administration

QBS72S should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Administer the initial QBS72S infusion over one hour. If tolerated, the team may administer subsequent infusions over 30 minutes. Administer QBS72S intravenously via central line or port. If no central line or port is available, the team may use a peripheral line to administer. Following administration, flush the line with 10 to 20 mL of normal saline at the end of the infusion. Administer using PVC tubing with a 0.2-micron filter. Additional information about administration can be found in the Pharmacy Manual.

4.1.3. Dose

QBS72S will be administered at 18 mg/m², or at 12 mg/m² for dose-reduced participants. For additional information on dose reduction, see Section 5.6 Dose Modifications and Dose Limiting Toxicities.

4.1.4. Regulatory Status of QBS72S

QBS72S is not approved for marketing in the United States for any indication, and all uses are investigational. Regulatory authorization to conduct this study is pursuant to IND 158711 submitted by Stanford investigator Melanie Hayden Gephart, MD, MAS. In addition, Quadriga Biosciences, Inc. has submitted a Letter of Authorization of Cross-reference to their IND 146107 on behalf of this study.

4.1.5. QBS72S Preparation, Handling, Storage, and Accountability

4.1.5.1. Acquisition and Accountability

The PI will be responsible for the documentation of lot numbers, dose and administration records and storage of QBS72S, and is described in detail in the CMC documentation. The PI will be responsible for ensuring study drug is correctly labeled and the appropriate dose provided per the protocol.

QBS72S will be ordered per Section 4.1.6.1 Investigational Agent Ordering and shipped to Stanford Investigational Drug Service (IDS). IDS will dispose of any expired or unused product.

4.1.5.2. Formulation, Appearance, Packaging, and Labeling

QBS72S is packaged by the company Societal CDMO, Inc. as a sterile lyophilized product prepared for injection with 50 mg extractable product per vial. The contents of each 20 mL glass vial are a white to off white powder or cake. The volume of the solution in the vial provides for an extractable volume of 10 mL that can be withdrawn by syringe. The vial is designed for single use.

QBS72S is a cytotoxic drug. Follow institutional handling and disposal procedures for cytotoxic drugs.

See Pharmacy Manual for further details.

4.1.5.3. Storage and Stability

QBS72S should be refrigerated at 2 to 8 °C.

Stability studies have demonstrated that QBS72S is stable for at least 6 months. These stability studies are ongoing.

See the Pharmacy Manual for storage conditions of the study intervention once reconstituted and/or diluted.

4.1.5.4. Preparation

Use normal saline solution (0.9% Sodium Chloride Injection, USP) to reconstitute QBS72S lyophilized powder and make a 50 mg/10 mL (5 mg/mL) nominal concentration of QBS72S. Further use normal saline solution (0.9% Sodium Chloride Injection, USP) to dilute reconstituted QBS72S (5 mg/mL) to the final concentration for infusion. See Pharmacy Manual for further details.

4.1.6. Investigational Agent Supply

The company Societal CDMO, Inc. manufactures the supply of QBS72S for this study. The point of contact for ordering QBS72S is Trinh Le; trinh@ccrc-inc.com.

4.1.6.1. Investigational Agent Ordering

Quadriga Biosciences will arrange delivery of QBS72S to Stanford, to be stored at the Stanford Health Care (SHC) Investigational Drug Services (IDS) under a material transfer agreement.

The IDS will be responsible for storage and dispensing of the drug following a standard institutional process after the participant is screened and enrolled on the study.

Questions about agent ordering can be directed to:

Aaron Weitzman, MD
Quadriga Biosciences
339 S. San Antonio Road, Suite 2A
Los Altos, CA 94022
650-416-4575
ron.weitzman@quadrigabiosciences.com

The interventional agent will be delivered to and managed by the Stanford Investigative Drug Service (IDS).

5. TREATMENT PLAN

5.1. Treatment Assignment

After the participant is determined to be completely eligible to participate in the study, the OnCore sequence number, a unique participant identifier, will be sequentially assigned. All participants successfully enrolled to the study will receive study drug QBS72S. There is no blinding or randomization.

5.2. Treatment Dose

QBS72S will be administered initially at a single dose of 18 mg/m² to all enrolled participants. This is based on the highest approved dose cleared by the Phase I clinical trial Cohort Review Committee. Participants may be lowered to a reduced dose of 12 mg/m². Please see Section 5.6 Dose Modification for details.

5.3. Treatment Schedule

QBS72S will be administered once a cycle, or every 28 days $\pm$ 2 days in Cycles 1 and 2, and every 28 days $\pm$ 3 days in Cycles 3 and beyond.

5.4. Treatment Administration Restrictions

A dose of study treatment at Cycle 1 may only be given if the participant meets all eligibility criteria (see Section 3.1 Eligibility Criteria and Participant Eligibility Checklist). A dose of study treatment Cycle 2 and beyond may be given only if:

- ANC $\geq$ 1,000/mm³.
- Platelet count $\geq$ 75,000/ mm³.

Non-hematologic toxicities have returned to baseline or Grade $\leq$ 1 severity (except for alopecia, nausea, and vomiting) or, at the Investigator discretion, Grade $\leq$ 2 if not considered a safety risk for the participant.

5.5. Treatment Duration

All participants regardless of LAT1 expression profile will continue to receive infusions of QBS72S until the participant experiences unacceptable toxicity or the participant refuses to continue, whichever occurs first. Refer to Section 5.8 Criteria for Removing Participants from Study Intervention and/or Study for reasons a participant may be withdrawn or discontinued from study participation.

5.6. Dose Modifications and Dose-Limiting Toxicities

Participants experiencing a dose-limiting toxicity (DLT) will have their dose delayed until either treatment is resumed or the participant is withdrawn from active study treatment. Treatment may be resumed at the lowered dose of 12 mg/m².

Adverse event documentation and reporting is described in Section 7 Adverse Events and Reporting Procedures.

Every effort should be made to administer study treatment on the planned dose and schedule. In the event of significant toxicity, dosing may be delayed as described below. In the event of multiple toxicities, dose modification should be based on the worst toxicity observed.

Participants are to be instructed to notify Investigators at the first occurrence of any adverse symptom.

Participants may be lowered to a reduced dose of 12 mg/m² if they experience intolerable toxicity or infusion reaction despite supportive care, but there is evidence of potential clinical benefit to continuing.

Within a dosing cycle, the dose may be interrupted until adequate recovery during a given treatment cycle.

Between dosing cycles, the next cycle administration may be delayed due to persisting toxicity when a new cycle is due to start. Additionally, the planned dose may be lowered from 18 mg/m² to 12 mg/m² or raised from 12 to 18 mg/m² at least 3 days in advance of the planned infusion.

5.6.1. Dose Discontinuation

Participants experiencing Grade 3 or 4 infusion reaction will immediately have their treatment halted and will be removed from study without re-challenging.

5.6.2. Dose Interruptions and Delays

If a participant experiences a Grade 3 or Grade 4 potentially treatment-related toxicity or experiences an intolerable Grade 2 toxicity despite supportive care during administration of QBS72S, then administration will be interrupted and the treating Investigator consulted. If the toxicity resolves after dose interruption, the treating Investigator will determine whether the participant may complete the remaining QBS72S dose. If the treating Investigator assesses that the toxicity is not or will not resolve to an acceptable level, QBS72S dose administration will be suspended. Appropriate follow-up assessments will be done until adequate recovery occurs as assessed by the Investigator. A participant may go on to receive future doses of QBS72S after

discussion with the PI if there is documentation of clinical benefit following recovery of the AE to acceptable levels as judged by the Investigator.

If QBS72S administration is suspended and not re-started the same day:

- If the toxicity resolves to an acceptable level as judged by the Investigator within 5 calendar days, the participant may receive the remaining amount of QBS72S that was not administered following interruption.
- If the toxicity resolves to an acceptable level as judged by the Investigator between 6 and 27 calendar days following dose interruption, the participant will receive their next full dose at their next regularly scheduled Day 1 of the following treatment cycle.

If the toxicity resolves to an acceptable level as judged by the 28 calendar days or more following dose interruption, then treatment will be resumed immediately and the day when treatment is restarted will be counted as Day 1 of a new dosing cycle. Subsequent cycles will be scheduled based on the start date of the new dosing cycle.

5.6.3. Potential Adverse Events

A set of non-GLP and GLP toxicology studies has been performed with QBS72S. Repeat dose toxicity studies and toxicokinetic evaluation followed by a 4-week recovery period were conducted in Sprague Dawley rats and cynomolgus monkeys. The overall adverse reactions observed in the presence of QBS72S are generally consistent with the published findings with other alkylating agents, e.g., (melphalan HCl (Evomela). The key potential target organs for QBS72S include: the bone marrow (e.g., hematological changes) and gastrointestinal tract (e.g., watery feces, weight loss) and skin ulcer, all of which are potentially relevant to humans. Notably for Evomela the warnings and precautions include:

- Gastrointestinal toxicity: Nausea, vomiting, diarrhea, or oral mucositis may occur; provide supportive care using antiemetic and anti-diarrheal medications as needed
 - Anti-Nausea and Anti-Diarrheal Medication: Primary prophylaxis is at the Investigator's discretion. The choice of the prophylactic drug is up to the Investigator as well as the duration of treatment, assuming there is no known or expected drug-drug interaction.

The most common adverse reactions observed in at least 50% of patients treated with melphalan are neutrophil count decreased, WBC count decreased, lymphocyte count decreased, platelet count decreased, diarrhea, nausea, fatigue, hypokalemia, anemia, and vomiting. The following AEs would be expected with QBS72S:

- Bone Marrow Suppression: Periodic complete blood counts will be monitored, and supportive care will be provided for infections, symptomatic anemia and bleeding until there is adequate hematopoietic recovery.
 - Infection Prophylaxis: Participants, at the discretion of the Investigator, will be given anti-infective agents as needed. Prophylaxis can begin at the time of screening and

- can continue for up to 1 month post the last QBS72S administration or longer if CD4 counts are < 200 cells/ μ L.
- Herpes Virus and Epstein Barr Virus: Participants can receive valacyclovir orally or acyclovir intravenously if participant cannot take oral medications.
 - Pneumocystis: Participants can receive regimens of doses of sulfamethoxazole + trimethoprim as fixed combination beginning at the start of screening. Pentamidine may be substituted in participants with a sulfa allergy.
 - Fungal: Fluconazole can be initiated and given either orally or as an IV dose. It should be continued until the ANC is > 1000 cell/ mm^3 .
 - Gastrointestinal Toxicity: Nausea, vomiting, mucositis, and diarrhea may occur. Use of prophylactic antiemetic medications will be allowed and supportive care will be provided for nausea, vomiting, diarrhea, and mucositis. Including nutritional support and analgesics for participants with severe mucositis.
 - Hepatotoxicity: Liver chemistries will be monitored, and supportive care provided for symptomatic hepatic disorders including such as hepatitis and jaundice.
 - Hypersensitivity: Acute hypersensitivity reactions, including anaphylaxis, may occur (e.g. urticaria, pruritus, edema, and skin rashes, tachycardia, bronchospasm, dyspnea, and hypotension.) supportive care will be provided for the reaction and QBS72S will be discontinued for serious hypersensitivity reactions.
 - Embryo-Fetal Toxicity: Based on its mechanism of action, QBS72S: can cause fetal harm when administered to a pregnant woman. Pregnant women will be excluded from this protocol.
 - Infertility: Based on its mechanism of action, QBS72S: Can cause suppression of ovarian function in premenopausal women resulting in persistent amenorrhea. Based on its mechanism of action QBS72S: can cause reversible or irreversible testicular suppression.
 - Infusion Related Reactions: Infusion reactions may occur with QBS72S administration. Infusion related reactions are typically characterized by fever, chills (rigor) and less commonly, hypotension. If this is observed, prophylactic medication will be administered to reduce the symptoms for subsequent doses. It is recommended that participants be pretreated with acetaminophen (650-1000 mg) and diphenhydramine (25-50 mg) approximately 30 minutes prior to the next dose. Two additional doses of acetaminophen may be given every 4-6 hours as needed. However, the Investigator should follow institutional practices if the local standard of care is different. All pretreatment and other medications given must be recorded in the participant's eCRF.
 - Tumor Lysis Syndrome (TLS): TLS occurs when tumor cells release their content in the blood stream, either spontaneously or due to treatment, leading to metabolic disturbances including hyperuricemia, hyperkalemia, hypophosphatemia and hypocalcemia. The incidence and severity of TLS depends on tumor volume, potential

for the tumor to lyse, and participant characteristics. Preservation of kidney function is most important and management of cardiac and neuromuscular abnormalities. Allopurinol can be used to reduce the hyperuricemia and rasburicase can be used to preserve or improve renal function.

- Because hyperkalemia can cause sudden death due to cardiac arrhythmias, participants should limit potassium and phosphorus intake during the TLS risk period (0-3 months).
- Frequent measurements of serum electrolytes, continuous cardiac monitoring will occur, and the administration of oral sodium polystyrene will be provided as appropriate. Symptomatic hypocalcemia will be treated with calcium supplementation; non-symptomatic hypocalcemia will not require treatment.

Results from the Phase I trial in Australia are still being analyzed, but initial reports are in line with the information listed above.

As of September 2023, one participant out of the eleven enrolled and treated so far has experienced a Suspected Unexpected Serious Adverse Event (SUSAR). The participant experienced unexpectedly severe cerebral edema and was hospitalized. The participant had previously received two rounds of Whole Brain Radiation (WBRT), which their care team also believes contributed to the event. Their cerebral edema never rose to a life-threatening level, and they were hospitalized as soon as it was detected. They improved with additional steroids and were discharged from the hospital two weeks after admission. Investigators and the hospital care team believe that the etiology of the cerebral edema was a combination of factors: potential treatment effect from QBS72S, progression of intracranial metastases, and some level of drug toxicity.

Treatment effect is expected in all drugs that are successfully cytotoxic, and most of the time has no symptoms. In this study's participant population, we expect some participants to experience headaches, nausea, vomiting, lethargy, weakness, or a new neurologic symptom depending on where and how bad the swelling is. In severe cases, brain swelling can lead to death.

More detailed information about the known and expected benefits and risks and reasonably expected AEs of QBS72S may be found in the Investigator's Brochure which is the single reference safety document (SRSD) for this study. The Investigator's Brochure is expected to be updated with the information about the above-described SUSAR in the spring of 2024.

5.7. Concomitant Medications, Procedures, and Supportive Care Guidelines

Concomitant treatment considered necessary for the participant may be given at discretion of the treating physician as part of their normal clinical management. All concomitant treatments, blood products, as well as nondrug interventions received by participants from screening until the end of study visit will be recorded on the eCRF.

5.7.1. Premedication Guidelines

Premedication consistent with institutional guidelines is recommended for all participants. Baseline premedications and escalation guidelines for nausea and vomiting symptoms are below.

- All patients may take an antihistamine and antiemetic prior to each infusion. These medications may be self-administered. As an example, these drugs could include:
 - 25-50mg diphenhydramine either IV prior to infusion or PO 1-2 hours before infusion as a self-administered medication.
 - 8mg ondansetron PO 1-2 hours before infusion as a self-administered medication, then 8-16mg ondansetron via IV as needed during infusion, and then participants are sent home from their visit with 8mg ondansetron to take every 8 hours as needed.
- Escalation Step 1: Consider adding 4-10 mg dexamethasone IV as needed, or PO 4-10mg dexamethasone 1-2 hours before infusion as a self-administered medication prior to each infusion.
- Escalation Step 2: Consider adding 1-2mg lorazepam IV prior to infusion.
- Escalation Step 3: Consider adding 125 mg aprepitant PO 1 hour before infusion as a self-administered medication. Then consider prescribing 80 mg orally once a day in the morning for two days following the infusion as a self-administered medication.

5.7.2. Supportive Care

Palliative and supportive care for disease-related symptoms may be administered at the Investigator's discretion and according to any available American Society of Clinical Oncology guidelines.

Participants currently being treated with a gonadotropin-releasing hormone agonist (GnRH agonist) may continue treatment while on clinical study as long as the GnRH agonist treatment has been well tolerated for at least 3 months prior to study entry.

Limited exposure/duration radiation therapy to treat pain is permitted. Palliative radiotherapy to the CNS or other body sites is permitted with permission from the overall study PI. Generally, study treatment should not be given within 7 days before or after radiotherapy.

5.7.3. Hematopoietic Growth Factors

Primary prophylactic use of granulocyte-colony stimulating factors is not permitted prior to first dose, but they may be used to treat treatment-emergent neutropenia as indicated by the current American Society of Clinical Oncology guidelines [24].

Erythropoietin may be used at the Investigator's discretion for the supportive treatment of anemia.

5.7.4. **Corticosteroids**

Chronic systemic corticosteroid use for palliative or supportive purposes is permitted. Prophylactic administration prior to chemotherapy administration will be allowed per standard of care. Acute emergency administration corticosteroids, and acute and chronic topical applications, inhaled sprays, eye drops, or local injections of corticosteroids are allowed. Participants requiring corticosteroids must be on a dose equivalent of dexamethasone 8 mg daily or less that has been stable or decreasing for ≥ 5 days prior to each brain MRI assessment after C1D1, or obtain written PI approval.

5.7.5. **Permitted Medications**

The following medications are permitted at the discretion of the Investigator:

- H2-Receptor Antagonists for symptomatic treatment of gastrointestinal disorder (including, but not limited to famotidine, ranitidine, nizatidine).
- Local antacids for symptomatic treatment of gastrointestinal disorder (e.g., aluminum/calcium hydroxide, aluminum/calcium carbonate, bismuth subsalicylate).
- Acid reducing agents (including proton pump inhibitors) can be used for symptomatic treatment of gastrointestinal disorder.
- 5-HT₃ receptor antagonist can be used for the symptomatic treatment of gastrointestinal disorder (including but not limited to ondansetron or granisetron).

5.7.6. **Prohibited Concomitant Medications**

Participants may not use or have an anticipated need for the following medications while in the treatment phase of the study:

- Anti-cancer therapies, including chemotherapy or immunotherapy not explicitly allowed by this protocol
- Bisphosphonates
- EIAEDs including: phenytoin, phenobarbital, carbamazepine, fosphenytoin, primidone, and oxcarbazepine
- Live vaccines
- Natalizumab
- Nivolumab
- Ocrelizumab
- Palifermin
- Pimecrolimus
- Tacrolimus
- Tofacitinib

- Antioxidant dietary supplements, vitamins, or herbal supplements.

5.8. Criteria for Removing Participants from Study Intervention and/or Study

Participants are free to withdraw consent and discontinue participation in a study at any time without prejudice to further treatment. The investigator may discontinue a participant from treatment intervention or the entire study for medical or administrative reasons. The following reasons may lead to discontinuation from the study intervention or the entire study.

- There is need for any treatment not allowed by the protocol (See Section 3.1 Eligibility Criteria and Section 5.7.6 Prohibited Concomitant Medication)
- Disease recurrence or progression, unless presence of reasonable evidence of clinical benefit and absence of any relevant toxicity
- Toxicity precluding further study treatment
- Study non-compliance
- Participant becomes pregnant
- Participant refuses further treatment or follow-up
- Withdrawal of consent (See Section 5.8.1 Withdrawal of Consent)
- Participant is lost to follow-up (See Section 5.8.2 Lost to Follow-Up)
- Investigator discretion

5.8.1. Withdrawal of Consent

Participants who request to discontinue receipt of study treatment will remain in the study and must continue to be followed for protocol-specified follow-up procedures unless they specifically withdraw consent to follow up. The withdrawal of consent should be explained in detail in the medical records by the Investigator, as to whether the withdrawal is only from further receipt of IP or also from study procedures and/or post-treatment study follow-up. If vital status (whether the participant is alive or dead) is being measured, publicly available information should be used to determine vital status only as appropriately directed in accordance with local law.

If a participant withdraws from the study, they may request destruction of any remaining samples, but data already generated from the samples will continue to be available and may be used to protect the integrity of existing analyses. The Investigator must document any such requests in the site study records. If the participant withdraws from the study and also withdraws consent for disclosure of future information, no further evaluations will be performed, and no additional data will be collected. Stanford may retain and continue to use any data collected before such withdrawal of consent.

5.8.2. Lost to Follow-Up

A participant will be considered lost to follow-up if they repeatedly fail to return for scheduled visits and is unable to be contacted by the study site.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The care team will attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether the participant wishes to and/or should continue in the study
- Before a participant is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the participant. Where possible, three separate contact attempts should be documented in the participant's medical record
- Should the participant continue to be unreachable, they will be considered to have withdrawn from the study.

5.9. Duration of Follow-Up

After completion of treatment (i.e., date of last treatment), participants will be followed until death. If a participant is removed from study due to unacceptable adverse event(s) or toxicity, that participant will be followed and safety data collected for at least 30 days and until resolution or stabilization of the adverse event, whichever is longest, to the extent possible, and then followed in the long term follow up phase until death. Participants that personally withdraw will be requested to attend a 30-day follow-up visit (or longer), or otherwise receive a follow-up contact, unless specifically countermanded by the participant.

5.10. Study Completion or Discontinuation

The study will reach primary completion when the last of the ORR study data, including all follow-up, is expected to be collected, i.e., all enrolled participants have either withdrawn or received six cycles of QBS72S.

The study will reach total completion when the last study data, including all follow-up data, has been collected, and analysis is complete. This is expected to occur when all participant data has been analyzed per Section 10 Statistics.

No participant data will be collected once the study is complete per this section.

Conditions may be discovered during the conduct of the study by the investigator, regulatory authorities, or other oversight bodies that indicate that the study should be terminated prematurely. The reasons that may warrant termination include, but are not limited to:

- The study is determined to be non-feasible, including inadequate accrual
- Determination of unexpected, significant, and/or unacceptable risk to participants
- Demonstration of efficacy that would warrant termination, investigator decision

- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility
- Business decision by the Principal Investigator, institution, or funding source, etc.

Written notification will be provided by the investigator to the funding entities, and regulatory authorities as appropriate, including the IRB of record.

6. STUDY PROCEDURES AND ASSESSMENTS

6.1. Schedule of Events

Study Phase	Screening ¹	Treatment ²								End of Treatment ³	Safety Follow Up Visit ⁴	Long Term Follow-Up ⁵
Day of Phase	-28 to -1	Cycle 1				Cycle 2		Cycle 3+				
		D1	D8	D15	D22	D1	D15	D1	D15			
Allowed Time Window		± 2	± 2	± 2	± 2	± 2	± 2	± 3	± 3	± 7	± 7	± 21
Study Day	-28 to -1	1	8	15	22	29	36					
QBS72S infusion ⁶		X				X		X				
Premedication ⁷		X				X		X				
Informed consent	X											
Demographics	X											
Medical history	X											
Complete physical exam ⁸	X					X				X		
Limited physical exam ⁸		X	X	X	X		X	X	X			
Neurological exam	X					X		X		X		X ²⁰
Vital signs ⁹	X	2X	X	X	X	2X	X	2X	X	X		
Height	X											
Weight	X	X	X	X	X	X	X	X	X	X		
Quality of life exam ¹⁰	X	X				X		X		X		
Performance status (KPS)	X	X				X		X		X		
ECG	X -14 to -1									X		
Hematology ¹¹ and Chemistry ¹²	X -14 to -1	X	X	X	X	X	X	X	X	X		
Coagulation ¹³	X -14 to -1					X		X		X		
Urinalysis ¹⁴	X -14 to -1					X		X		X		
Pregnancy testing ¹⁵	X -7 to -1	X				X		X		X		
MRI ¹⁶	X -14 to -1	-----See Section 6.2.1.1. Imaging Timing-----								X		X ²⁰
Systemic imaging ¹⁷	X	-----See Section 6.2.1.1. Imaging Timing-----								X		X ²⁰
Pharmacokinetics LMD only ¹⁸		2X										
Biomarker sample LMD only ¹⁹		2X	X	X	X	X	X	X	X	X		X ²⁰
Adverse events		-----X-----								X	X	
Con meds and procedures	X	-----X-----								X	X	
Survival follow-up												X q8w ±21d

¹ Screening must occur within 28 days of Day 1. Assessments obtained within 4 days of Day 1 do not have to be repeated on Day 1. Some assessments have a narrower screening window as shown in the table.

² Assessments shown for Cycle 3 apply to all subsequent cycles.

³ The end-of-treatment (EOT) visit should occur ≤7 days after the decision to discontinue study participation. The EOT visit may occur later based on PI discretion, such as prolonged hospitalization. The visit at which MRI showed progressive disease may be used as the EOT visit provided that all required assessments were performed. MRI does not have to be repeated if performed ≤ 14 days or subject has documented initial progression. ECG does not have to be repeated if performed ≤14 days. If the visit

occurs via telemedicine, only KPS and limited physical (as allowable over video) are required. EOT visits that occur via telemedicine will not be considered protocol deviations.

- ⁴ All participants who discontinue study treatment will have a safety follow-up visit to collect AEs and SAEs that may have occurred. This visit will occur approximately 30 (± 7) days after the EOT visit. This visit may occur in person, via telephone, or via video call.
- ⁵ All participants who discontinue study treatment will be followed for survival and further anti-cancer therapy information following their last dose. This follow-up will occur every 8 weeks ± 3 weeks beginning after their safety follow-up visit. This may occur in person or via telephone, video call, or chart review.
- ⁶ QBS72S Infusion: Initially QBS72S will be administered intravenously over 1 hour (± 10 minutes) every 28 days (± 2 days) beginning Cycle 1 Day 1. The infusion may be decreased to a minimum of 30 minutes if preferred.
- ⁷ See Section 5.7.1 Premedication Guidelines.
- ⁸ A complete physical examination may include an evaluation of head, eyes, ears, nose and throat, neck, heart, chest (including lungs), abdomen, extremities and/or musculoskeletal, skin, lymph nodes, cardio-vascular status and neurological status. A limited physical examination should be directed at the evaluation of symptoms or specific safety issues. Changes from baseline abnormalities should be recorded at each subsequent physical examination. A complete physical exam will be conducted at screening, C2D1, and at in-person EOT visits.
- ⁹ Vital signs: systolic and diastolic blood pressure [SBP and DBP], pulse rate, oral, temporal or tympanic temperature, and respiratory rate. For non-infusion visits, collect vitals at any point in the visit. On infusion visits for all participants, collect vitals pre-dose and 15 minutes (± 7 minutes) following the end of infusion.
- ¹⁰ Quality of life will be assessed via EORTC QLQ-C30 and BN20 assessments. See Section 6.2.2.6 Quality of Life Exam and Appendix B for more information.
- ¹¹ A complete blood count (CBC) includes hemoglobin, hematocrit, platelet count, red blood cell (RBC) count and white blood cell (WBC) count with differential. Results must be obtained during screening ≤ 14 days prior to C1D1.
- ¹² Chemistry includes albumin, alkaline phosphatase, AST, ALT, bicarbonate, blood urea nitrogen (BUN), calcium, chloride, creatinine, fasting glucose, LDH, magnesium, phosphate, potassium, sodium, total bilirubin, total protein, and uric acid. Results must be obtained during screening ≤ 14 days prior to C1D1.
- ¹³ Coagulation labs are INR, PT, and PTT. Results must be obtained during screening ≤ 14 days prior to C1D1.
- ¹⁴ Urinalysis will be assessed using urine dipstick. Urine microscopy will be performed if urine dipstick is abnormal. Urinalysis includes pH, glucose, protein, ketones, bilirubin, blood, and specific gravity. If urine protein is $\geq 2+$ by dipstick, a 24-hour urine for total protein and a random urine for total protein and creatinine will be obtained and evaluated. Results must be obtained during screening ≤ 14 days prior to C1D1.
- ¹⁵ For participants of childbearing potential, a serum pregnancy test must be obtained during screening ≤ 7 days prior to C1D1. For subsequent pregnancy testing on study and at end of treatment, urine pregnancy tests are allowed. If a urine pregnancy test is positive, a confirmatory serum pregnancy test is required.
- ¹⁶ Tumors will be assessed by brain MRI during screening ≤ 14 days prior to C1D1, then every 6 weeks ± 7 days per institutional guidelines, and lastly as part of the EOT visit. Refer to Section 6.2.1.2 Imaging Tumor Evaluation and Response Assessment.
- ¹⁷ Systemic disease imaging must be performed for all participants, whether or not they have known systemic disease. Imaging will include any other areas of known disease that are being measured. Refer to Section 6.2.1 Efficacy Assessments.

- ¹⁸ Pharmacokinetic serum and CSF samples will be collected once before the start of the infusion and once following the end of the infusion, with no other time restrictions. PK samples will be collected from Cohort 2 and Cohort 3 participants only. See Section 8.2 Pharmacokinetics (PKs).
- ¹⁹ Biomarker serum and CSF samples may be collected at every Cohort 2 and Cohort 3 participant's in-person visit to Stanford's Palo Alto location. See Section 8.3 Correlative Study of Biomarkers.
- ²⁰ These assessments will occur in long term follow-up approximately every 8 weeks (± 21 days) or per SOC as long as is feasible.

6.2. Description of Procedures and Assessments

6.2.1. Efficacy Assessments

6.2.1.1. Imaging Timing

A brain MRI with and without contrast (brain MRI) will be collected for each participant at the following timepoints: up to 14 days prior to C1D1, 6 weeks following C1D1 (± 14 days), every 6 weeks following (± 14 days) while on active treatment, and for the EOT visit. The EOT brain MRI can be collected up to four weeks prior to the date of the EOT visit and up to four weeks following. If a brain MRI on active portion falls within window for the EOT timepoint, no duplicate scan will be collected.

Note that there may be 10 weeks between the brain MRI collected in screening and the first brain MRI collection following administration of IP: 14 days screening + 6 weeks post C1D1 + 14 days collection window.

Systemic imaging will be collected for each participant up to 6 weeks prior to C1D1. A CT CAP is the preferred systemic imaging type during screening. Following screening, the research team physician coordinating care for the participant will determine the appropriate type and frequency of systemic imaging. Generally, if systemic disease is noted during screening, the site or sites of systemic disease will be imaged 6 weeks following C1D1 (± 14 days), every 6 weeks following (± 14 days) while on active treatment, and for the EOT visit. EOT systemic imaging for participants with noted systemic disease can be collected up to four weeks prior to the date of the EOT visit and up to four weeks following. If a systemic imaging timepoint from active portion falls within window for the EOT timepoint, no duplicate scan will be collected.

Note that there may be up to 14 weeks between the systemic imaging collected in screening and the first systemic imaging collection following administration of IP: 6 weeks screening + 6 weeks post C1D1 + 14 days collection window.

If systemic disease is not noted on systemic imaging at screening, systemic imaging will be repeated every 3 months following C1D1 while on active treatment, and optionally for the EOT visit. Optional EOT systemic imaging for participants with no noted systemic disease can be collected up to six weeks prior to the date of the EOT visit and up to six weeks following. If a brain MRI on active portion falls within window for the optional EOT timepoint, no duplicate scan will be collected.

If a group other than the study team orders either a CT CAP or a skull to thigh PET CTs, data from these scans can be used in place of the study-ordered systemic imaging for any timepoint. If a group other than the study team orders a brain MRI with and without contrast, data from this scan can be used in place of the study-ordered brain MRI for any timepoint.

Said another way, any existing imaging that falls within a study timepoint window should be used instead of ordering repeat imaging specifically for the study. For example, if the participant with noted systemic disease receives C1D1 on 01-Jan and receives a skull to thigh PET CT on 12-Feb, the study team will not order additional imaging during the 5-Feb to 19-Feb window.

If an external group orders an MRI or systemic imaging that does not fall within a usable timepoint window, the next imaging timepoint will be calculated based on the external group imaging date. Said another way, if imaging is performed out of window, the clock for future imaging is reset and new dates are calculated to avoid too-frequent scans. For example, if the participant with noted systemic disease receives C1D1 on 01-Jan and receives a CT CAP ordered by a non-study team clinician on 20-Jan, the study team will next order systemic imaging between 24-Feb and 10-Mar.

6.2.1.2. Imaging Tumor Evaluation and Response Assessment

Imaging will be read by Stanford Radiology team and provided to clinical team per Stanford's standard of care. As part of the screening brain MRI, index lesions will be identified by the PI or Sub-I which will be used to evaluate treatment response throughout the study. Screenshots of these lesions, including the series number and image number, must be included in the participant's electronic medical record prior to the first infusion. Any MRIs performed following EOT per SOC will be additionally collected as available. Data from any clinically-indicated additional systemic scans will be collected, including imaging of the spine in participants with LMD.

Anti-tumor activity will be assessed through radiological tumor assessments conducted per institutional guidelines at screening, during treatment, whenever disease progression is suspected (e.g., symptomatic deterioration), and at the time of withdrawal from treatment (if not done in the previous four weeks). Assessment of response will be made using the modified Response Assessment in Neuro-oncology Brain Metastases (mRANO-BM) criteria (see Section 10.2.1.2). Efficacy evaluations using the mRANO-BM criteria involve post-contrast MRI findings, use of corticosteroids, and neurological exam results [36].

6.2.2. Safety and Other Assessments

6.2.2.1. Demographic and Medical History

Medical history, date of birth, age (calculated), sex, ethnicity, and race will be recorded at screening. The medical history assessment will include:

- History of breast cancer, including diagnosis dates of primary disease, parenchymal disease, leptomeningeal disease, and any active systemic disease. Where possible, confirm biomarker positivity values, see below.
- Any diagnosis in the past 2 years or any ongoing diagnosis. Relevant ongoing medical history will be graded as per CTCAE version 5.0.
- History of disease process (e.g., staging) other than the cancer under study (active or resolved) and concurrent illness.

- Date of diagnosis and information on prior anti-tumor treatments, surgeries, radiotherapy, and recurrence date.
- Any baseline signs and symptoms experienced within 14 days prior to C1D1.

Medical occurrences that begin before the start of study intervention but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions (including prior non-medications, procedures and surgeries) section of the eCRF, not the AE section.

Where possible, the study team will confirm breast cancer receptor status. For the purposes of this protocol, triple-negative breast cancer (TNBC) is defined as 5% or less expression of ER receptor, 5% or less expression of PR receptor, and either a HER2 receptor negative FISH result or a HER2 receptor IHC result of 0 or 1+ [45, 46, 51]. These criteria are based on meetings held by the Stanford Cancer Institute Breast Oncology Group with participation from Michelle Melisko, MD (UCSF), in which it was discussed that there is a subset of patients who have low receptor positivity but otherwise their cancer behaves like TNBC. 2020 ASCO/CAP guidelines [52] define TNBC as including ER-negative if 0% or < 1% because recent data suggest there is limited efficacy of hormonal treatment in a subset of “low-positive” patients. At low levels of expression, the response to hormonal therapy is heterogeneous, suggesting these tumors may act more like TNBC.

Additionally, for the purposes of this protocol, ER positivity is defined as over 5% expression of ER receptor (6% or higher), and PR positivity is defined as over 5% expression of PR receptor (6% or higher). HER2 positivity is defined as either a HER2 receptor positive FISH result or a HER2 receptor IHC result of higher than 1 [45, 46, 51].

6.2.2.2. Physical Examinations

Complete physical examinations and symptom-directed physical examinations will be performed by a licensed physician at the time points specified in the study schedules.

Complete physical examinations may include examination of general appearance, head, ears, eyes, nose, throat, dentition, thyroid, chest (heart, lungs), abdomen, skin, neurological, extremities and/or musculoskeletal system, back, neck, musculoskeletal, and lymph nodes.

Symptom-directed physical examinations will follow clinical signs related to previous serious illnesses and participant direction. Physical examinations may be performed at various unscheduled time points if deemed necessary by the Investigator.

6.2.2.3. Neurological Exam

Institutional standard of care neurologic examinations may include assessments of:

- Response to auditory and/or tactile stimulus
- Pupillary response to light
- Strength of hand grip and movement of extremities bilaterally
- Ability to sense touch/pain in extremities.
- Mental Status

- Cranial Nerves
- Motor Exam
- Reflexes
- Gait

6.2.2.4. Vital Signs

Oral or tympanic temperature, pulse rate, respiratory rate, and systolic and diastolic blood pressure will be assessed.

6.2.2.5. Weight and Height

Body height and body weight will be collected and used to calculate BMI. BMI is calculated by dividing the participant's body weight in kilograms by the participant's height in meters squared (kg/m^2). If a patient is physically incapable of standing and their weight is not collected, this will not be considered a protocol deviation.

6.2.2.6. Quality of Life Exam

Quality of life will be assessed via the EORTC QLQ-C30 and BN20 measurements [42, 43, 44]. Both questionnaires will be completed by participants in person every 4 weeks, coinciding with their in-person infusion visit. Both questionnaires will also be completed by participants at their end of treatment (EOT) visit. If the EOT visit is completed via telehealth, the Quality of Life questionnaires are optional.

As July 2021, EORTC QLQ-C30 and BN20 are available to the study team in English and Spanish (Mexico localization). If participants with a different first language enroll, every effort will be made to work with EORTC to obtain a set of questionnaires in the participant's native language. Additional languages will be added to Appendix B in future amendments per the needs of future participants. If the EORTC is unable to provide a set of questionnaires in the participant's chosen language, a translator may be used to proxy-score the English set of questionnaires on the participant's behalf.

6.2.2.7. Performance Status

Performance status will be measured on the Karnofsky Performance Status (KPS) scale per Table 4 [20, 21]. The KPS scale is an assessment tool for functional impairment. It can be used to compare effectiveness of different therapies and to assess the prognosis in individual participants. In most serious illnesses, the lower the KPS score, the worse the likelihood of survival.

Table 4. Karnofsky Performance Status Scale

Condition	Value	Level of Functional Capacity
	100	Normal, no complaints, no evidence of disease
	90	Able to carry on normal activity

Able to carry on normal activity and to work; no special care needed	80	Normal activity with effort
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed	70	Cares for self, unable to carry on normal activity or to do active work
	60	Requires occasional assistance but is able to care for most personal needs
	50	Requires considerable assistance and frequent medical care
Unable to care for self; requires equivalent of institutional or hospital care; diseases may be progressing rapidly	40	Disabled; requires special care and assistance
	30	Severely disabled; hospital admission indicated although death not imminent
	20	Very sick; hospital admission necessary; active supportive treatment necessary
	10	Moribund; fatal processes progressing rapidly

6.2.2.8. ECGs

12-lead ECG tracing (with a 10-second rhythm strip) will be obtained. All ECG tracings will be reviewed by the PI or a Sub-I within 5 business days, ideally day-of or the day following. When the timing of these measurements coincides with a blood collection, the ECG should be obtained prior to the nominal time of the blood collection, BP, and pulse rate.

6.2.2.9. Lab Values

Lab values to collect:

- CBC with differential: hemoglobin, hematocrit, platelet count, red blood cell (RBC) count and white blood cell (WBC) count with differential.
- Chemistry: albumin, alkaline phosphatase, AST, ALT, bicarbonate, blood urea nitrogen (BUN), calcium, chloride, creatinine, fasting glucose, LDH, magnesium, phosphate, potassium, sodium, total bilirubin, total protein, and uric acid.
- Coagulation labs: INR, PT, and PTT
- Urinalysis: pH, glucose, protein, ketones, bilirubin, blood, and specific gravity
 - Urinalysis will be assessed using urine dipstick. Urine microscopy will be performed if urine dipstick is abnormal. If urine protein is $\geq 2+$ by dipstick, a 24-hour urine for total protein and a random urine for total protein and creatinine will be obtained and evaluated.

All normal values will be determined by Stanford laboratory's published normal value ranges at time of evaluation.

6.2.2.10. Systemic Imaging

Systemic disease imaging at baseline will include any other areas of known disease that are being measured. After screening, areas of active disease noted at screening will be imaged per standard of care timelines. Assessment of response will be made using the RECIST 1.1 criteria

(see Section 10.2.1.2). Efficacy evaluations using the RECIST 1.1 criteria involve clinical imaging measurements [23].

6.2.2.11. Concomitant Medications and Procedures

Concomitant medications are relevant medications taken during screening period until the follow-up/EOS visit or up to a 30-day follow-up period if the medication was taken for AEs deemed related to treatment.

Restrictions relating to concomitant medication use are outlined in Section 5.7 Concomitant Medications, Procedures, and Supportive Care Guidelines.

Prior therapy or concomitant therapy (after study drug administration) with any medications, including both prescription and non-prescription drugs should be discussed with the Investigator before study drug administration, except in the case of necessary treatment of AEs or where appropriate medical care necessitates that therapy should begin before the Investigator can consult with the PI.

All medications, including over the counter medications, vitamins, and herbal supplements, taken by participants during the course of the study will be recorded in the eCRF and coded using the most current WHO drug dictionary available at the commencement of the study. Concomitant medications will be listed by participant and summarized by anatomical therapeutic chemical and preferred name.

Concomitant procedures are procedures that are performed with the intent of addressing active disease state from the time of screening to the End of Treatment visit. This study will only record concomitant procedures that pertain to breast to brain parenchyma metastases, breast to brain LMD, or active sites of breast cancer systemic disease noted at screening. Concomitant procedures will be recorded by associated procedure code, name of procedure, date, time, and target anatomic location of procedure.

6.2.2.12. Survival Follow Up

Researchers will check each participant's survival status every 8 weeks (± 21 days). This may be conducted through chart review of recent clinical visits or through phone call. If a participant withdraws consent or is lost to follow-up (See Section 5.8.1 Criteria for Removing Participants from Study Intervention and/or Study), only publicly available survival information will be used.

7. ADVERSE EVENTS AND REPORTING PROCEDURES

7.1. Adverse Event Definitions

An adverse event (AE) is any untoward medical occurrence in a patient or clinical study participant, temporally associated with the use of study intervention, whether or not considered related to the study intervention.

Serious adverse events (SAEs) are defined per the FDA definition at [21CFR§312.32\(a\)](#) and [ICH GCP E6](#). An adverse event is considered “serious” if, in the opinion of the Protocol Director or a sub-investigator, it results in ANY of the following.

- Death
- Life-threatening adverse event with an actual and immediate risk of death [21CFR§312.32(a)]
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Event jeopardizes the participant and may require medical or surgical intervention to prevent one of the outcomes listed above

7.2. Classification of Adverse Events

7.2.1. Severity of Event

NCI CTCAE version 5.0 is used to assess the severity of adverse events in this study. [22]

7.2.2. Adverse Event Attribution to Intervention or Study

AEs, serious or otherwise, will be attributed by the Principal Investigator or qualified designate to study treatment in accordance with the definitions below.

• Definitely Related. The event follows a reasonable temporal sequence from administration of the study drug, the event follows a known response pattern to the study drug, and, based on the known pharmacology of the study drug, the event is clearly related to the effect of the study drug. • Probably Related. The event follows a reasonable temporal sequence from administration of the study drug, and the event follows a known response pattern to the study drug, and the event cannot be reasonably explained by an intercurrent medical condition, or the event cannot be the effect of a concomitant medication. • Potentially / Possibly Related. The event follows a reasonable temporal sequence from administration of the study drug or the event follows a known response pattern to the study drug, but the event could have been produced by a concomitant medication or an intercurrent medical condition, which, based on the pathophysiology of the condition and the pharmacology of the study drug, is unlikely to be related to the use of the study drug.	These are treated as “ Related ”
• Unlikely to be related. The event is clearly due to causes distinct from the use of the study drug, such as a documented pre-existing condition, the effect of a concomitant medication, or a new condition that, based on the pathophysiology of the condition and the pharmacology of the study drug, is unlikely to be related to the use of the study drug. • Not Related. A causal relationship between the study treatment and the AE is not a reasonable possibility.	These are treated as “ Not Related ”

7.2.3. Expectedness of Event

The Principal Investigator or qualified designate will also assess all recorded adverse events on basis of event severity, frequency (if applicable/assessable), and the established product risk information described within the product Investigator Brochure; and/or this protocol document, as to whether the events are “expected” or “not expected” relative to the study interventions and/or the study / procedures.

Note that unexpected adverse events may have reporting requirements as described elsewhere in this section.

7.2.4. Time Period of Event Assessment and Follow-Up

All AEs will be followed until resolution or until at least 30 days after the last dose of the study treatment.

7.3. Potential Adverse Events and Risks

In addition to the summary risks described in Section 1.3 Potential Risks and Benefits, adverse events described by severity and frequency within the research agent QBS72S Investigator Brochure are considered anticipated, i.e., not unexpected.

7.4. Adverse Event Monitoring and Collection

Untoward medical events experienced by a study participant receiving an interventional drug, device, or procedure, or within 30 days after such treatment, will be considered an AE, regardless of whether or not considered drug-related.

Abnormal laboratory results constitute adverse events only if requiring therapeutic intervention (e.g., low hemoglobin that requires transfusion) or an action be taken with the study drug (e.g. drug held, reduced or discontinued). Abnormal laboratory results designated Not Clinically Significant (NCS) are captured as lab value data, see Section 6.2.2.9 Lab Values and will not be duplicated as AEs.

7.4.1. Case Report Forms for Adverse Event Reporting

Both SAEs and non-serious adverse events will be described in source documentation and listed on study-specific Case Report Forms (CRFs or eCRFs).

All adverse events should be recorded, regardless of relationship, except as described in this document. A single “overarching” diagnosis should be entered instead of observed signs, symptoms, and significant laboratory findings.

Any pre-existing condition that worsens in severity/Grade or frequency should be recorded as a new adverse event, except as described in this document.

7.5. Adverse Events of Special Consideration

7.5.1. Progressive Disease

Disease progression, including disease progression that results in death, is part of the natural course of the disease under study. Therefore, it is not considered an AE/SAE that needs to be reported, unless the investigator considers that the event is related to study treatment.

7.6. Adverse Event Reporting

Reportable AEs, based on relatedness (attribution) to the study agent; expectedness, severity (Grade 1 to 5), seriousness (Yes/No), or any other aspect of the investigation, will be reported as described below.

- SAEs will be reported to the Quadriga BioSciences Inc. via e-mail to trinh@ccrc-inc.com
- SAEs will be reported to the SCI Data and Safety Monitoring Committee (DSMC) by submission to CCTO-Safety.
- SAEs will be reported by the IND-holder to the IND under which this study is being conducted within 7 days (for a life-threatening or fatal event) or 15 days (for other SAEs) of the IND-holder's determination that the SAE meets the criteria for an IND safety report, i.e., the event was serious, unexpected, and at least possibly-related to the study drug [a serious, unexpected, suspected adverse reaction (SUSAR)]. IND Safety Reports will be submitted to the IND using the MedWatch Form FDA 3500A for mandatory reporting.
- All **Unanticipated Problem (UPs)** associated with the use of a drug, biologic, or device will be reported as follows.
 - To Stanford's IRB, by the Stanford Protocol Director as specified by the IRB document "Events and Information that Require Prompt Reporting to the IRB" (GUI-P13).
 - To the SCI DSMC, by submission to CCTO-Safety / OnCore as an SAE.

Subsequent follow-up reports, whether to SAEs or UPs, will be reported as required by the receiving entities.

All SAEs will be followed until resolution or until stable, whichever is longer.

7.7. Adverse Event Records

The investigator will retain adverse event source data, supporting documentation of attribution and seriousness, and copies of official adverse event reports or SAE CRFs.

8. CORRELATIVE AND SPECIAL STUDIES

8.1. Sample Coding for Privacy Protection

A laboratory code will be generated for each study sample generated. The codes will be generated in sequential order based on collection order, for example Q201-099 for study participant 99. Study participant identity cannot be ascertained from the code.

Participant data will be stored in HIPAA-compliant eCRF database REDCap under participant MRN and name in an encrypted password protected computer.

8.2. Pharmacokinetics (PKs)

8.2.1. PK Background

Cohort 2 and Cohort 3 participants will be fitted with Ommaya reservoirs for collecting CSF, which is standard practice for LMD patients. To ascertain the level of QBS72S that is bioavailable in the CSF following infusion, amounts of QBS72S in each sample will be measured by mass spectrometry using standard techniques already developed by Quadriga, and brain exposure of QBS72S will be evaluated by comparing serum levels to CSF levels [37].

8.2.2. PK Collection and Handling

Pharmacokinetic (PK) specimen collection will involve Cohort 2 and Cohort 3 participants only. These participants will have Ommaya reservoirs placed per standard of care practice. One sample of up to 10ccs of CSF can be drawn following every Cohort 2 or Cohort 3 participant's C1D1 infusion. Additionally, up to 10ccs of peripheral blood will be drawn during a Cohort 2 or Cohort 3 participant's C1D1 visit with no further time restrictions. Failure to collect blood or CSF at any study timepoint is not considered a protocol deviation, as the safety and appropriateness of drawing blood or drawing CSF from an Ommaya reservoir at a visit is up to the discretion of the treating Investigator.

CSF samples will be separated into cell-free and cell pellet portions, and blood samples will be separated into cell and plasma components. Samples will then be aliquotted and labelled. Aliquots will be preserved by snap-freezing at -80°C. For additional details, please consult the Alliance Pharma Biospecimen Laboratory Manual.

Specimens will be batch-shipped to Quadriga Biosciences once all specimens have been collected. Specimens will be shipped at -80°C.

8.2.3. Sites Performing Correlative Study

Quadriga Biosciences is a privately held pharmaceutical company developing proprietary targeted cancer therapies. They are located at 339 S. San Antonio Road, Suite 2A, Los Altos, CA 94022; 650-917-9098; info@quadrigabiosciences.com.

8.3. Correlative Study of Biomarkers

8.3.1. Biomarkers Background

Leptomeningeal disease cannot be biopsied due to the diffuse nature of the disease and high surgical morbidity. The two current gold standard methods for detecting LMD are MRI and CSF cytology. However, both of these methods are notoriously insensitive [40, 41] and are inadequate for tracking responses to therapy. For example, the number of metastatic cancer cells in CSF is very low, on average from 1 to 100 cells/mL. CSF molecular biomarkers potentially offer a much more sensitive and powerful way to assess treatment response in LMD.

8.3.2. Biomarker Specimen Collection and Handling

For participants in Cohort 2 and Cohort 3, up to 30 ccs of CSF and up to 20 mL peripheral venous blood may be collected at all in-person visits to Stanford where possible. Failure to collect blood or CSF at any study timepoint is not considered a protocol deviation, as the safety and appropriateness of drawing blood or drawing CSF from an Ommaya reservoir at a visit is up to the discretion of the treating Investigator.

CSF samples will be separated into cell-free and cell pellet portions, and blood samples will be separated into cell and plasma components. Samples will then be aliquotted and labelled. Aliquots will be preserved by snap-freezing at -80°C. For additional details, please consult the Hayden Gephart Laboratory Manual.

All biomarker samples will be analyzed at Stanford's Palo Alto location at Dr. Hayden Gephart's lab, located at 1201 Welch Road, Palo Alto, CA 94305.

8.4. Correlative Study of Archival Tumor Tissue Specimens

8.4.1. Archival Tumor Biopsy Background

QBS72S was developed to leverage high expression of the transporter LAT1 within both the blood brain barrier and within tumor cells. LAT1 is overexpressed in most tumors, regardless of tissue of origin. Because it is an underpinning mechanic, there is a possible correlation between the metastatic cell line's relative expression of LAT1 and the efficacy of treatment. The primary cancer cell line's relative expression of LAT1 is much easier for clinicians to assess and has a high likelihood of reflecting the metastatic cell line's expression, so is used instead.

The study team will also explore biomarkers related to TMZ treatment and PARP inhibitors found in the archival tumor biopsy tissue for areas of future research interest.

8.4.2. Archival Tissue Collection and Handling

Archival tumor tissue specimens will be collected for every participant through Stanford's Neuropathology department. Researchers will request either paraffin-embedded tissue blocks or at least 8 slides of each participant's primary breast tumor and parenchymal brain metastases as available through Stanford Neuropathology's previously established research guidelines. Additionally, any fresh, flash-frozen tissue samples will be requested if available.

Archival tumor tissue specimens will be transported to Dr. Hayden Gephart's laboratory space, located at 1201 Welch Road Palo Alto CA 94305, for sequencing and LAT1 expression testing.

8.4.3. Archival Tissue Analysis

Samples will be analyzed via IHC in the Hayden Gephart Lab. Additionally, IHC analysis will be independently verified by a board-certified neuropathologist.

All biomarker samples will be analyzed at Stanford's Palo Alto location at Dr. Hayden Gephart's lab, located at 1201 Welch Road, Palo Alto, CA 94305.

9. REGULATORY CONSIDERATIONS AND DATA REPORTING

9.1. Review and Approval of Protocol by IRB and SCI SRC

This protocol, the proposed informed consent, and all forms of information related to the study that will be provided to the participants (e.g., questionnaires, handouts, written instructions, diaries, advertisements used to recruit participants, etc.) will be submitted to, reviewed, and approved by the SCI Scientific Review Committee (SRC) and the Stanford Administrative Panels on Human Subjects in Medical Research of the Research Compliance Office (i.e., the Stanford IRB) (the "IRB of record") prior to initiation of the research. Any changes made to the protocol will be submitted as a modification and will be approved by the IRB of record prior to implementation.

This study will be conducted in accordance with the iteration of the protocol that is currently IRB-approved.

9.2. Protocol Compliance and Deviations

9.2.1. Compliance with the Protocol

No deviation or changes from the procedures and process described by the IRB-approved protocol, except those necessary to eliminate an immediate hazard(s) to trial participants, or when the change(s) involves only logistical or administrative aspects of the trial [e.g., change in study monitor(s), change of telephone number(s)], will be knowingly permitted without review and approval by the IRB of record.

9.2.2. Protocol Deviations

Any deviation (or violation) from the IRB-approved protocol, including those that eliminate an immediate hazard or are administrative in nature, will be documented and explained in the study site file. All deviations at the Stanford clinical site that meet the reporting requirements will be reported to the Stanford IRB by the Stanford investigator within the defined timeframes. All deviations will be reported to the Stanford IRB either annually in the IRB continuing review, or individual as IRB Prompt Reports.

For all studies monitored by the SCI Data and Safety Monitoring Committee (DSMC) as defined in Section 9.3 Data and Safety Monitoring Plan, all deviations, exceptions, and/or violations of the protocol, as well as deviations, exceptions, and/or violations to applicable IRB policies and

overarching regulations, will be reported to the SCI data Safety and Monitoring Committee (DSMC). Accordingly, as the DSMC only reviews reports recorded in OnCore, such events will be promptly submitted in the OnCore record for the study, per the current SCI DSMC SOP.

9.3. Data and Safety Monitoring Plan

The Principal Investigator is responsible for monitoring the conduct of the study including oversight of safety and protocol compliance. On an ongoing basis, the Principal Investigator will review safety data and identify any changes to the research necessary to ensure the appropriate measures and monitoring necessary for participant safety. In addition to the Principal Investigator's safety monitoring role, the SCI DSMC will conduct data and safety monitoring activities for this study.

9.3.1. Monitoring

In addition to investigator self-monitoring (Section 9.3.1.1), the SCI DSMC will monitor study-related activities to determine whether the study has been conducted in accordance with the protocol, local standard operating procedures, FDA regulations, [GCP, and SOPs](#). In addition, the DSMC will regularly review serious adverse events and protocol deviations associated with the research to ensure the protection of human participants. Results of DSMC monitoring activities will be communicated to the investigator, who has the responsibility to provide such reports to the IRB of record and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as needed.

9.3.1.1. Investigator Monitoring

Pursuant to the Guideline for [GCP](#), monitoring is defined as the act of overseeing the progress of a clinical trial, and of ensuring that it is conducted, recorded, and reported in accordance with the protocol, standard operating procedures (SOPs), GCP, and the applicable regulatory requirement(s).

The Principal Investigator is responsible for self-monitoring the progress of the trial on a continuous basis. Monitoring may be delegated to an appropriately-trained individual. Self-monitoring includes, but is not limited to, those items that will be reviewed during an audit (see also Section 9.5 FDA Oversight). Routine monitoring by the Principal Investigator will be conducted at an estimated frequency of not less than twice a year.

9.4. Data Management

Source documents for all research data will be retained in accordance with all applicable regulations and institutional requirements for data retention. These materials will be made available for monitoring and/or auditing by SCI DSMC, other monitoring body and/or regulatory agencies.

9.4.1. Data Management Plan

Coordinators will input study data into the REDCap eCRF database within 7 business days of receipt by the study team; best practice will be to enter data within 2-3 days of receipt.

All study data will be stored in HIPAA-compliant eCRF database REDCap under participant MRN and name in an encrypted password protected computer. This will be linked to the actual participant sample by a code number, not related to PHI. The research coordinator will be responsible for database records of participant data. For the laboratory personnel, the samples will have a unique code which can be tracked back to this PHI database only by study protocol staff with the appropriate training in the handling of PHI. Participant charts may be reviewed by Stanford PI and Study Coordinator for yearly audits. Database lock will be performed 3 years after permanent closure of enrollment.

The clinical research team will have access to the secure, password-protected database linking laboratory code numbers to PHI. Electronic files will be kept on locked, encrypted Stanford computers with password protected files. Paper files, including a Participant Identification Log, will be kept onsite at Stanford's Cancer Clinical Trials Office (CCTO). The minimum necessary number of personnel will have access to this PHI. All research team members are HIPAA trained and will attend regular sessions focused on the responsible conduct of research for human participants and the Collaborative Institutional Training Initiative (CITI) Program.

This study will work with the CCTO's Data Safety Monitoring Committee to ensure adequate and thorough capture and review. The Stanford Principal Investigator will additionally provide a data monitoring plan describing how and when the Stanford Principal Investigator and/or designates will monitor the study. The monitoring will be adequate to assure that the valid consent is obtained and documented; the per-protocol data is collected; records and databases are maintained with adequate and accurate participant case histories; adverse events are reported; clinical protocol is maintained; and the study is conducted according to the established procedures of the IRB of record. Original source documents include clinical charts and hospital, laboratory, and pharmacy records.

9.5. FDA Oversight

9.5.1. Investigational New Drug (IND) Application Considerations

This single-center study will be conducted under IND-158711, held by Dr. Melanie Hayden Gephart at Stanford University.

Per FDA's input, it is understood that that the time-to-event endpoints in single-arm trials, such as the secondary endpoint of progression-free survival (PFS) in this trial, will not be interpretable from a regulatory standpoint.

9.5.1.1. Protocol Amendments

The protocol will be conducted at all times according to the current version of the protocol as approved by the IRB of record. IRB-approved protocol versions will be submitted to the IND as a Protocol Amendment: Change in Protocol.

9.5.1.2. IND Reports

Information regarding the progress and status of this study will be submitted to IND-158711, in accordance to the content and format defined at [21CFR§312.32 \(Safety Reports\)](#) and [312.33 \(Annual Reports\)](#).

10. STATISTICAL CONSIDERATIONS

10.1. Statistical Plan

The study will employ the Simon's 2-stage design [24], which is one of the most common multi-stage designs used in Phase 2a clinical trials that allows flexibility regarding the null and alternative hypotheses while also allowing stopping for futility. The null hypothesis that the true response rate is 10% will be tested against a one-sided alternative. For definition of primary objective evaluable participants from Cohort 1, see Section 10.2.1.1 Relevant Subset for Primary Objective. In the first stage, 15 primary objective evaluable participants will be accrued and treated with QBS72S. If there are one or fewer responses in these 15 participants, the study will be stopped. Otherwise, 10 additional evaluable participants will be accrued for a total of 25 evaluable participants. The null hypothesis will be rejected if six or more responses are observed in these 25 participants. This design yields a type I error rate of <5% and power of 80% when the true response rate is 30% (see Table 5).

Table 5. Study Design Considerations to Ensure Adequate Power

n	n₁	R₁	R₂	Type 1 Error	Power	EN₀	Probability of early stopping
25	15	1	5	0.0328	0.8017	19.5	0.5490

n is the total number of subjects

n₁ is the number of subjects accrued during stage 1

r₁, if **r₁** or fewer responses are observed during stage 1, the trial is stopped early for futility

r₂, if **r₂** or fewer responses are observed by the end of stage two, then no further investigation of the drug is warranted

EN₀ is the expected sample size for the trial when response rate is *p*₀

All participants regardless of LAT1 expression profile will continue to receive treatment every four weeks until disease progression, unacceptable toxicity, withdrawal from the study, or the clinician's decision to conclude treatment. The study will track participant clinical outcomes until death.

10.1.1. Sample Size Determination

Sample sizes were arrived at by combining potential participant streams from UCSF and Stanford. Researchers consulted Stanford Medicine's STARR database of archival patient visits to Stanford Cancer Center's Neuro-Oncology and Neuro-Radiology clinics for incidence of potentially eligible participants from 2008 to 2018, then added UCSF's breast oncology department potential participant estimates. This combined tally was then lowered to account for 50% of potentially eligible participants choosing to pursue other options.

10.1.2. Minimization of Bias

All participants will receive the same study intervention, and there is no blinding or randomization. We will track race and ethnicity and assess any differences in clinical outcomes as part of our secondary analyses. We will not exclude any groups by sex, gender, orientation, race, or ethnicity. Participants will be identified by their treating physicians at Stanford or referring sites (oncology, radiation oncology, neuro-oncology, and neurosurgery) via MRI of the brain with contrast. This study will be listed on the Stanford Clinical Trials website and

Clinicaltrials.gov. Research study staff will not approach potential participants for recruitment until gaining explicit permission from the potential participant's treating physician. Study information, such as study title, contact information, and summary of inclusion and exclusion criteria, will be posted on the departmental website. The PI or research staff will discuss the study with the participant during a normal clinic visit or with a visit scheduled for the purpose of recruitment to the study.

10.2. Study Endpoints

10.2.1. Primary Objective

The primary objective is overall response rate (ORR). This objective is not assessing a safety issue.

10.2.1.1. Relevant Subset for Primary Objective

ORR will be measured using only data from Cohort 1 (Stages 1+2). Primary objective evaluable participants from Cohort 1 (Stages 1+2) are defined as all of: demonstrating high to moderate LAT1 expression, receiving baseline MRI at screening, at least one complete dose of QBS72S at 18 mg/m², and one additional MRI scan following receipt of at least one dose of QBS72S. For identifying high to moderate LAT1 expression in participant archival tissue via IHC, see Stanford Hayden Gephart Laboratory Manual. Participants that qualify as non-evaluable under this definition will be replaced.

10.2.1.2. Endpoint Definition for Primary Objective

ORR will be measured using modified Response Assessment in Neuro-Oncology for Brain Metastases (mRANO-BM) and RECIST 1.1 response criteria [36, 23]. ORR will be measured by response rate across primary objective evaluable participants in Cohort 1 (Stages 1+2). A successful response is defined as ≥30% decrease in sum total diameter of all target lesions compared with baseline across two or more sets of scans, provided that steroid dose is lower or the same as baseline and neurological evaluations are stable or improved. See Table 6 and further descriptions below for additional detail.

Each participant will have their lesions indexed and measured at baseline for maximum diameter, with a minimum of one to a maximum of five target lesions, and a minimum size for each lesion being 3mm or greater at baseline. These lesions are designated as target lesions. The number of target lesions may increase during data collection if one or more new lesions measuring at least 3mm or greater develops while the participant is on study. Response is broken down into the following categories: complete response, partial response, progressive disease, and stable disease. Both complete and partial response qualify as responses for the purposes of this study.

Table 6. Overall Response Rate Criteria

State of Disease	Change in Maximum Diameter of Lesion
Preliminary complete response	100% decrease

Confirmed complete response	100% decrease for 4+ weeks
Preliminary partial response	≥30% decrease
Confirmed partial response	≥30% decrease for 4+ weeks
Preliminary progressive disease	≥25% increase
Confirmed progressive disease	≥25% increase for 6+ weeks
Stable disease	Any response not complete, partial, or progressive
Pseudoresponse	Any preliminary complete or preliminary partial response that does not evolve into confirmed complete response or confirmed partial response on the following scan
Pseudoprogession	Any preliminary progressive disease that does not evolve into confirmed progressive disease on the following scan

Preliminary Complete Response is defined as 100% decrease in diameter of all target lesions compared to baseline on a single set of scans.

Confirmed Complete Response is defined as requiring all of the following conditions to be met:

1. A 100% decrease in sum total diameter of all target lesions compared with baseline, sustained across two sets of scans taken approximately 4 weeks apart. The first scan exhibiting a 100% decrease in diameter of all target lesions is considered preliminary complete response. If the second scan exhibits an increase in the sum total diameter of all target lesions with respect to the preliminary complete response scan, then the response is not sustained, this response is noted as pseudoresponse and is now considered preliminary progressive disease (see progressive disease section). If the second scan continues to exhibit 100% decrease in sum total diameter of all target lesions, and criteria 2 and 3 are met, it is considered a confirmed complete response and the participant should continue on therapy until confirmed progressive disease is observed. If criteria 2 and 3 are not met, consult with the *Stable Disease* and *Confirmed Progressive Disease* sections.
2. Steroid dose should be the same or lower compared with baseline scan.
3. Stable or improved neurological examinations.

Preliminary Partial Response is defined as a ≥30% decrease in sum total diameter of all target lesions compared with baseline on a single set of scans.

Confirmed Partial Response is defined as requiring all of the following conditions to be met:

1. A ≥30% decrease in sum total diameter of all target lesions compared with baseline, sustained across two sets of scans taken approximately 4 weeks apart. The first scan exhibiting a ≥30% decrease in sum of products of diameters of all target lesions compared with baseline is considered preliminary partial response. If the second scan exhibits less than a 30% increase in sum total diameter of all target lesions compared

with the preliminary partial response scan, then the response is not sustained, is noted as pseudoresponse, and is now considered preliminary progressive disease (see progressive disease section). If the second scan exhibits a $\geq 30\%$ increase in sum total diameter of all target lesions compared to the preliminary partial response scan, and criteria 2 and 3 are met, response is considered a confirmed partial response and the participant should continue on therapy until confirmed progressive disease is observed. If criteria 2 and 3 are not met, consult with the *Stable Disease* and *Confirmed Progressive Disease* sections.

2. Steroid dose should be the same or lower compared with baseline scan.
3. Stable or improved neurological examinations.

Preliminary Progressive Disease is defined as a $\geq 25\%$ increase in sum total diameter of all target lesions compared with baseline on a single set of scans.

Confirmed Progressive Disease is defined as requiring any of the following conditions to be met:

1. At least two sequential scans separated by 4 weeks, both exhibiting a $\geq 25\%$ increase in sum total diameter of all target lesions compared with baseline. The first scan exhibiting a $\geq 25\%$ increase in the sum total diameter of all target lesions compared with baseline should be compared to the smallest sum total diameter of all target lesions obtained either at baseline or at best response, whichever is smaller, and is noted as preliminary progressive disease. If the second scan at least 4 weeks later exhibits a $\geq 25\%$ increase in sum total diameter of all target lesions relative to baseline, it is considered confirmed progressive disease and the participant should discontinue therapy. If the second scan at least 4 weeks later exhibits a $< 25\%$ increase in sum total diameter of all target lesions relative to baseline, this scan showing preliminary progressive disease is noted as pseudoprogression and the participant should continue on therapy until a second increase in tumor size relative to the pseudoprogression scan is observed. Note that any new measurable ($\geq 3\text{mm}$) enhancing lesions will not be immediately considered progressive disease, but instead will be added the target lesions going forward and will contribute to the sum total diameter of all target lesions. This definition will be applied to all analyses.
2. Clear clinical deterioration not attributable to other causes apart from tumor (e.g., seizures, medication adverse effects, therapy complications, stroke, infection) or attributable to changes in steroid dose.
3. Failure to return for evaluation as a result of death or deteriorating condition.

Stable disease is defined as requiring all of the following conditions to be met:

1. Does not qualify for confirmed complete response, confirmed partial response, or confirmed disease progression as defined above. Note this also applies to participants that demonstrate pseudoprogression when the confirmation scan does not show progressive disease or pseudoresponse when the confirmation scan does not show confirmed partial or total response.

2. In the event that corticosteroid dose was increased without confirmation of disease progression on neuroimaging, and subsequent follow-up imaging shows that the steroid increase was required because of disease progression, the last scan considered to show stable disease will be the scan obtained when the corticosteroid dose was equivalent to the baseline dose.

Pseudoresponse is defined as any preliminary complete or preliminary partial response that does not evolve into confirmed complete response or confirmed partial response on the following scan.

Pseudoproggression is defined as any preliminary progressive disease that does not evolve into confirmed progressive disease on the following scan.

Baseline is defined as the last available non-missing value collected prior to the first administration of IP. This definition will be applied to all analyses. Change from baseline values will be calculated as the difference between the result at a specific timepoint, and the baseline value for the specific endpoint. Percentage change from baseline values will be calculated as the ratio of the change from baseline value to the baseline value, expressed as a percentage. All available data will be used. No safety or efficacy data will be imputed; missing values will be treated as missing.

10.2.1.3. **Assessment Methods for Primary Objective**

Percentage growth or reduction in sum total diameter of target lesions will be measured by comparing baseline MRI tumor measurements to MRI tumor measurements taken at each timepoint following C1D1 until participant is removed from study.

MRI tumor measurements will be collected at Stanford's Palo Alto MRI facilities using Stanford's standard of care brain tumor scan protocols. These measurements are taken by Stanford board-certified radiologists independent from the study. This will hold true for all MRI measurements in this analysis.

10.2.1.4. **Measurement Time Points for Primary Objective**

The primary objective of overall response rate will be measured once all evaluable participants within the Cohort 1 Stage 1 have completed active participation in the study. Data included will be baseline, 1-month, 2-month, 3-month, 4-month, 5-month, and 6-month data collection events. Overall response rate will be measured again if Cohort 1 Stage 2 is enrolled to successfully once all participants have completed active participation.

10.2.1.5. **Response Review**

The primary objective of the overall response rate will be reviewed by radiologist Dr. Michael Iv. The review will consist of simultaneous review of the participant's files and radiological images. This review will occur once all expected participants have completed the active portion of the protocol.

10.2.2. Secondary Objectives

The secondary objectives will be measured through progression free survival, overall survival, and durations of response in participants with parenchymal metastases. A safety event listing will be provided for all participants in all cohorts.

10.2.2.1. Relevant Subset for Secondary Objectives

Progression-free survival, overall survival, durations of response, and a safety event listing will all be measured using data from all Cohort 1 (Stages 1+2) participants, regardless of primary objective evaluability.

10.2.2.2. Endpoint Definition for Secondary Objectives

Progression free survival is defined as the length of time that participants do not experience confirmed disease progression measured from the date of diagnosis of their study disease to the date of confirmed disease progression.

Overall survival is defined as the length of time that participants survive from the date of diagnosis of their study disease to the date of their death. For all participants who die, we will attempt to determine the cause of death. Participants will be considered to have died of neurologic causes if they had stable systemic disease and progressive neurologic dysfunction, as determined by a study-associated physician. Participants with severe neurologic disability who die of intercurrent illness will also be included among neurologic deaths, as will participants with both rapidly progressive systemic disease and advancing neurologic dysfunction. The systemic cancer will be considered the only cause of death if, in the setting of neurologic improvement or stabilization, participants develop fatal infections, hemorrhages, or failure of vital organ systems other than the brain. Participants whose deaths cannot be determined to be either neurologic or systemic will be classified as unknown [47].

Durations of response will be divided into two measures, DoR_{prog} and DOR_{DOD} . DOR_{prog} is defined as the length of time participants do not experience confirmed disease progression measured from the date of the first administration of QBS72S to the date of confirmed disease progression. DOR_{DOD} is defined as the length of time participants do not experience confirmed disease progression measured from the date of the first administration of QBS72S to the date the participants passes away.

Safety is defined as a listing of all adverse events, graded according to CTCAE v5.0. In addition, actual time on treatment in days will be measured.

10.2.2.3. Assessment Methods for Secondary Objectives

The date of diagnoses will be collected from participants during the screening process. The date of first dosage with QBS72S will be a recorded date collected in the CRFs. Date of progression will be determined by either the PI or one of the research physicians and will be recorded in the CRFs. Survival data will be collected through long term follow-up visits, communication with the participant's medical teams, and publicly available death data. The adverse event listing will use CTCAE v5.0 to grade all adverse events.

10.2.2.4. Measurement Time Points for Secondary Objectives

Progression free survival will be measured from the date of diagnosis of study disease until disease progression is observed or the participant passes away. Overall survival will be measured from the date of diagnosis of study disease until the participant passes away. Durations of response divided into two: DoR_{prog} and DOR_{DOD}. DoR_{prog} will be measured from first dose of QBS72S until disease progression; DOR_{DOD} will be measured from first dose of QBS72S until the participant passes away. Adverse events and toxicities will be recorded as soon as they are reported. Participants in follow up will be queried for possible toxicities.

10.2.3. Exploratory Objectives

The exploratory objectives are progression free survival, overall survival, and durations of response in Cohort 2 and Cohort 3; overall response rate, progression free survival, overall survival, and duration of response in participants with active systemic disease across all Cohorts; determining pharmacokinetics in CSF of QBS72S via PK analysis; exploring potential cellular and gene expression biomarkers; and exploring the relationship between LAT1 expression and response to treatment via IHC analysis of primary tumor.

10.2.3.1. Relevant Subset for Exploratory Objectives

Determining the PFS, OS, and DoR_{prog} and DoR_{DOD} will be measured using data from evaluable participants in Cohort 2 and Cohort 3. Determining the active systemic disease ORR, PFS, OS, and DoR will be measured using data from the subset of evaluable participants in Cohort 1 (Stages 1+2), Cohort 2, and Cohort 3 with actively monitored systemic disease sites besides parenchymal and leptomeningeal metastases. Determining the CNS distribution of QBS72S and exploring biomarkers will be measured using data from all Cohort 2 and Cohort 3 participants from whom CSF was collected and using data from matched control samples collected under Stanford Protocol IRB-12625 and utilized in Stanford Protocol IRB-34363. Determining the relationship between LAT1 expression and response will be measured using data from all participants in Cohort 1 (Stages 1+2), Cohort 2, and Cohort 3.

Exploratory objective evaluable participants from Cohort 2 and Cohort 3 are defined as demonstrating high to moderate LAT1 expression and having received at least one full 18mg/m² dose of QBS72S. For identifying high to moderate LAT1 expression in participant archival tissue via IHC, see Stanford Hayden Gephart Laboratory Manual. Participants that qualify as non-evaluable under this definition will be replaced.

10.2.3.2. Endpoint Definition for Exploratory Objectives

PFS is defined as the length of time that participants do not experience confirmed disease progression measured from the date of diagnosis of their study disease to the date of confirmed disease progression. Confirmed LMD disease progression will be determined by the scale found in Table 7. LMD diagnosis date is defined as the earliest positive date of evidence. Participants may have more than one score based on available evidence.

Table 7. Leptomeningeal Disease Progression Evidence Scale

Score	Evidence
6	LP result malignant
5	MRI confirmed LMD (obvious LMD development on MRI)
4	Treated for LMD: shunt, WBRT, or medication
3	LP result suspicious or atypical
2	MRI suspicious/suggestive, LP negative
1	Clinical signs/symptoms, neuro-oncologist suspicion
0	No LMD mentioned or seen

OS is defined as the length of time that participants survive from the date of diagnosis of their study disease to the date of their death. For all participants who die, we will attempt to determine the cause of death. Participants will be considered to have died of neurologic causes if they had stable systemic disease and progressive neurologic dysfunction, as determined by a study-associated physician. Participants with severe neurologic disability who die of intercurrent illness will also be included among neurologic deaths, as will participants with both rapidly progressive systemic disease and advancing neurologic dysfunction. The systemic cancer will be considered the only cause of death if, in the setting of neurologic improvement or stabilization, participants develop fatal infections, hemorrhages, or failure of vital organ systems other than the brain. Participants whose deaths cannot be determined to be either neurologic or systemic will be classified as unknown. [47]

Durations of response will be divided into two measures, DoR_{prog} and DOR_{DOD} . DOR_{prog} is defined as the length of time participants do not experience confirmed disease progression measured from the date of the first administration of QBS72S to the date of confirmed disease progression. DOR_{DOD} is defined as the length of time participants do not experience confirmed disease progression measured from the date of the first administration of QBS72S to the date the participant passes away.

Determining the CNS distribution of QBS72S will be measured by comparing blood serum and CSF levels of QBS72S to control data from Protocols IRB-12625 and IRB-34363. Biomarkers will be examined through extracting and analyzing freely circulating cells within the CSF and examining archival research tissue samples. Gene expression and cytological analyses of participant cells will be done to identify potential biomarkers of disease for future exploration. LAT1 expression profile of the primary tumor will be determined through IHC staining.

10.2.3.3. Assessment Methods for Exploratory Objectives

Determining CNS distribution of QBS72S will be analyzed through spectrophotometry. Relative levels in blood serum and in CSF will then be compared. Results will be expressed as the CSF to plasma drug ratio. Biomarkers in the CSF will be measured through expression and

cytological analyses, including RNA sequencing, cell-free RNA dynamic array sequencing, circulating tumor cell count, mutation analysis, and development of cell free nucleic acid paradigms. Results will be expressed as significant gene expression changes in participants with LMD.

10.2.3.4. Measurement Timepoints for Exploratory Objectives

For PK and biomarker measurement timing and procedures, see Section 8 Correlative and Special Studies.

10.3. Interim Analysis

The study will be stopped if there are one or fewer responses in the 15 participants enrolled in Cohort 1, Stage 1. The response for the purpose of the interim overall response rate will be evaluated for the first stage after the 15th participant completes 12 weeks of treatment and tumor growth is assessed. The overall response rate for the full study will be determined after the 25th participant completes 12 weeks of treatment and tumor growth is assessed. This stopping rule will ensure the given study design provides 80% power and 5% type I error rate.

10.4. Primary Analysis

The primary endpoint is the overall response rate (ORR) of enrolled participants. We are using modified Response Assessment in Neuro-oncology Brain Metastases (mRANO-BM) criteria to define response (see Section 10.2.1.2 Endpoint Definitions for Primary Objective). We will summarize ORR on all participants with a binomial proportion and estimate a 95% confidence interval using the Clopper-Pearson method. According to the Simon's two stage design (see Table 5), the null hypothesis will be rejected if six or more responses are observed in 25 evaluable participants. For a definition of primary analysis evaluable population, see Section 10.2.1.1 Relevant Subset for Primary Objective.

10.4.1. Primary Analysis Population

For the first stage, 15 evaluable participants from Cohort 1 Stage 1 will be enrolled. In case this study is continued for the second stage, 10 additional evaluable participants will be enrolled to Cohort 1 Stage 2. A total of 25 evaluable participants to be analyzed. For a definition of primary analysis evaluable population, see Section 10.2.1.1 Relevant Subset for Primary Objective.

10.4.2. Primary Analysis Plan

We will summarize ORR on all participants with a binomial response rate. The Clopper-Pearson [25] method will be used to estimate the two-sided 95% CI. The primary endpoint is the overall response rate (ORR) of enrolled participants. Descriptive statistics will include number of observations, mean, SD, median, range, and interquartile range for continuous valued variables, and the count and percentage for each level of categorical variables. 95% CIs will be presented where appropriate. Baseline is defined as the last available non-missing value collected.

Participant Disposition

The number and percentage of screened participants, enrolled participants, treated participants, participants discontinued from the study and study treatment, as well as the primary reason for discontinuation will be summarized using counts and percentages.

Demographics and Baseline Characteristics

Demographic characteristics including age, gender, race, and ethnicity and other baseline characteristics including weight, height, body mass index, and ECOG performance status will be summarized and listed.

Baseline disease characteristics and prior treatments including prior surgeries, radiotherapy and systemic therapies will be summarized and listed. General medical history will be summarized separately.

Medical history terms will be coded using the MedDRA version 22.0 or higher. Medical history will be summarized by MedDRA SOC and PT and reported as counts and percentages.

Extent of Exposure

The total number of cycles, dose administered per cycle and overall, as well as the duration of exposure will be calculated for each participant based on the collected study drug administration information and summarized by treatment.

Laboratory Results

Separate summaries and listings will be prepared for each laboratory test. All collected laboratory results, as well as the derived fields will be included in the data listings. A separate listing will be presented for participants with abnormal results.

10.5. Secondary Analysis

The objective of secondary analysis is to evaluate progression free survival, overall survival, durations of response, and safety in Cohort 1 (Stages 1+2) [30]. PFS, OS, and DoR will be expressed as numerical values without dispersion. Safety will be expressed as a list of adverse events.

10.5.1. Secondary Analysis Population

The secondary analysis population will include all participants enrolled in Cohort 1 (Stages 1+2), regardless of primary analysis evaluability.

The safety analysis set will include all participants who were enrolled into the study and who received the planned Cycle 1 dose of QBS72S, and either were assessed at both of the Cycle 1 safety assessments (Days 14 and 28) or withdrew due to treatment related toxicity. Participants not considered evaluable for safety will be replaced.

All baseline characteristics, safety, immunogenicity, and efficacy analyses will be conducted based on the safety analysis set.

10.5.2. Secondary Analysis Plan

Time to event distribution for progression free survival, overall survival, and duration of response will be estimated using Kaplan Meier techniques [26, 30]. Median survival time along with a 95% CI will be constructed [27, 28]. Rates at fixed time points will be derived from the Kaplan Meier estimate and corresponding confidence interval will be derived based on the Greenwood formula [29].

Participant Disposition

The number and percentage of screened participants, enrolled participants, treated participants, participants discontinued from the study and study treatment, as well as the primary reason for discontinuation will be summarized using counts and percentages.

Demographics and Baseline Characteristics

Demographic characteristics including age, gender, race, and ethnicity and other baseline characteristics, including weight, height, body mass index, and ECOG performance status. Will be summarized and listed.

Baseline disease characteristics and prior treatments including prior surgeries, radiotherapy and systemic therapies will be summarized and listed. General medical history will be summarized separately.

Medical history terms will be coded using the MedDRA version 22.0 or higher. Medical history will be summarized by MedDRA SOC and PT and reported as counts and percentages.

Extent of Exposure

The total number of cycles, dose administered per cycle and overall, as well as the duration of exposure will be calculated for each participant based on the collected study drug administration information and summarized by treatment.

Safety Analyses

The safety analysis set will be used for the analyses of safety and tolerability data. All safety variables will be summarized and listed.

Adverse Events

All AEs will be coded using the MedDRA. The Investigator will classify the severity of AEs using the NCI CTCAE v 5.0. An adverse event is defined as any event with an onset date on or after the date of first dose of study drug, or any event present before treatment that worsens after treatment. Only adverse events with an onset date prior to the date of last dose + 28 days will be tabulated in summary tables.

The number and percentage of participants who experience AEs will be summarized by SOC, PT, relationship to study drug, and severity for each treatment group. A by-participant listing will be provided for those participants who experience an SAE, including death, or experience an AE associated with early withdrawal from the study or discontinuation from study drug. Clinical laboratory data will be summarized by the type of laboratory test. The number and percentage

of participants who experience abnormal (i.e., outside of reference ranges) and/or clinically significant abnormalities after study drug administration will be presented for each clinical laboratory measurement. For each clinical laboratory measurement, descriptive statistics will be provided for baseline and all subsequent post-treatment scheduled visits. Changes from baseline to the post-treatment visits will also be provided. Descriptive statistics of vital signs will also be provided in a similar manner. In addition, shifts from baseline in CTCAE grade (where applicable) and by high/low flags (where CTCAE grades are not defined) will be presented by treatment.

Extent of Exposure

The total number of cycles, dose administered per cycle and overall, as well as the duration of exposure will be calculated for each participant based on the collected study drug administration information and summarized by treatment.

Laboratory Results

Separate summaries and listings will be prepared for each laboratory test. All collected laboratory results, as well as the derived fields will be included in the data listings. A separate listing will be presented for participants with abnormal results.

10.6. Exploratory Analysis

The objectives of the exploratory analysis are to:

- Evaluate the intra-cranial progression free survival, overall survival, and durations of response in Cohort 2 and Cohort 3.
- Evaluate the overall response rate, progression free survival, overall survival, and durations of response in systemic disease sites in participants treated with QBS72S in Cohort 1 (Stages 1+2), in Cohort 2, and in Cohort 3.
- Determine pharmacokinetics in CSF of QBS72S in Cohort 2 and in Cohort 3.
- Explore potential cellular and gene expression biomarkers of disease and therapeutic response in Cohort 1 (Stages 1+2), Cohort 2, and Cohort 3.
- Explore relationship between LAT1 expression and response to treatment via IHC analysis of primary tumor in Cohort 1 (Stages 1+2), Cohort 2, and Cohort 3.

10.6.1. Analysis Population for Exploratory Objectives

The exploratory analysis set will include all participants who were enrolled into Cohort 1 (Stages 1+2), Cohort 2, and Cohort 3 of the study and who received the planned Cycle 1 dose of QBS72S, and either were assessed at both of the Cycle 1 safety assessments (Days 14 and 28) or withdrew due to treatment related toxicity. Participants not considered evaluable for safety will be replaced.

All ORR, PFS, OS, DoR_{prog}, DoR_{DOD}, PK, biomarker analyses, and IHC will be conducted based on the exploratory analysis set.

10.6.2. Analysis Plan for Exploratory Objectives

ORR, PFS, OS, DoR_{prog}, and DoR_{DOD} will be analyzed in the sub-population of participants with systemic disease as described above. These will be expressed as numerical values without dispersion.

We will determine PK CNS distribution of QBS72S through spectrophotometry. Relative levels in blood serum and in CSF will then be compared [37]. Results will be expressed as the CSF to plasma drug ratio.

Biomarkers will be measured through circulating tumor cell count, mutation analysis, development of cell free nucleic acid paradigms, and expression and cytological analyses, including RNA sequencing and cell-free RNA dynamic array sequencing. We will conduct differential expression analysis between participant samples and control samples collected under Stanford Protocol IRB-12625 and utilized in Stanford Protocol IRB- 34363. Biomarkers will be expressed as statistically significant gene expression changes in LMD participants as compared to control participants from IRB-12625 and IRB-34363. These will be expressed as numerical values without dispersion.

LAT1 expression profile of the primary tumor will be determined through IHC staining of the primary tumor biopsy specimen, where available [46]. The IHC staining levels will be expressed as numerical values without dispersion.

10.7. Sample Size

10.7.1. Accrual Estimates

We expect to accrue 45 evaluable participants across all cohorts. We expect to accrue 15 evaluable participants for Cohort 1 Stage 1. We expect to accrue 10 evaluable participants for Cohort 1 Stage 2 for a total of 25 evaluable participants across the entirety of Cohort 1. In Cohort 2, we expect to accrue 10 evaluable participants. In Cohort 3, we expect to accrue 10 evaluable participants. For definitions of evaluability, refer to Section 10.2.1.1 Relevant Subset for Primary Objective and Section 10.2.3.1 Relevant Subset for Exploratory Objectives.

10.7.2. Sample Size Justification

The sample size was calculated for primary analysis for Cohort 1 (Stages 1+2). The null hypothesis that the true response rate is 10% will be tested against a one-sided alternative. In Cohort 1 Stage 1, 15 primary objective evaluable participants will be accrued and treated with QBS72S. If there are one or fewer responses in the 15 evaluable participants, the study will be stopped. Otherwise, 10 additional evaluable participants will be accrued to Cohort 1 Stage 2 for a total of 25 evaluable participants. For a definition of primary objective evaluable participants, see Section 10.2.1.1 Relevant Subset for Primary Objective. The null hypothesis will be rejected if six or more responses are observed in these 25 participants. This design yields a type I error rate of < 5% and power of 80% when the true response rate is 30% (see Table 5).

10.7.3. Effect Size Justification

We assumed a 10% response rate for the null hypothesis (i.e., under the no treatment) and a 30% rate response rate for the treatment effect for the power analysis. These estimates are based on recent clinical experience with mouse models at Stanford.

10.8. Criteria for Future Studies

If the primary endpoint is met, a multi-phase Phase III study in the target population will be launched. We will also explore additional Phase II studies in other breast cancer subtypes and non-breast primary tumors. If the primary endpoint is not met but we do succeed in meeting one or more secondary endpoints, we will explore additional Phase II studies in other breast cancer subtypes and non-breast primary tumors and analyze the responders to inform future cancer subtypes that may benefit.

10.9. Descriptive Statistics and Exploratory Data Analysis

Descriptive statistics will include number of observations, mean, SD, median, range, and interquartile range for continuous valued variables, and the count and percentage for each level of categorical variables. A 95% CIs will be presented where appropriate. Baseline is defined as the last available non-missing value collected. This definition will be applied to all analyses. All available data will be used. No safety or efficacy data will be imputed; missing values will be treated as missing.

11. REFERENCES

1. Zhang M, Sun H, Zhao S, *et al.* (2017). Expression of PD-L1 and prognosis in breast cancer: a meta-analysis. *Oncotarget*, 8(19), 31347–31354. <https://doi.org/10.18632/oncotarget.15532>
2. Hayashi K, Anzai N. (2017). Novel therapeutic approaches targeting L-type amino acid transporters for cancer treatment. *World journal of gastrointestinal oncology*, 9(1), 21–29. <https://doi.org/10.4251/wjgo.v9.i1.21>
3. American Brain Tumor Association. (2020). Metastatic Brain Tumors [Brochure]. Chicago, IL: Author. Retrieved 2021, from <https://www.abta.org/publications/metastatic-brain-tumors/>
4. Boyle P. (2012). Triple-negative breast cancer: epidemiological considerations and recommendations. *Annals of oncology: official journal of the European Society for Medical Oncology*, 23 Suppl 6, vi7–vi12. <https://doi.org/10.1093/annonc/mds187>
5. Surveillance, Epidemiology, and End Results Program. (n.d.). Cancer Stat Facts: Female Breast Cancer Subtypes (United States of America, National Institutes of Health, National Cancer Institute). <https://seer.cancer.gov/statfacts/html/breast-subtypes.html>
6. Lin NU, Claus E, Sohl J, Razzak AR, Arnaout A, Winer EP. (2008). Sites of distant recurrence and clinical outcomes in patients with metastatic triple-negative breast cancer: high incidence of central nervous system metastases. *Cancer*, 113(10), 2638–2645. <https://doi.org/10.1002/cncr.23930>
7. Martin AM, Cagney DN, Catalano PJ, *et al.* Brain Metastases in Newly Diagnosed Breast Cancer: A Population-Based Study. *JAMA Oncol.* 2017;3(8):1069–1077. doi:10.1001/jamaoncol.2017.0001
8. Sperduto PW, Mesko S, Li J, *et al.* (2020). Beyond an Updated Graded Prognostic Assessment (Breast GPA): A Prognostic index and trends in treatment and survival in breast Cancer Brain METASTASES from 1985 to today. *International Journal of Radiation Oncology*Biophysics*Physics*, 107(2), 334–343. doi:10.1016/j.ijrobp.2020.01.051
9. ClinicalTrials.gov Identifier: NCT04745975. Guided treatment based ON Mini-PDX in Metastatic triple negative breast cancer. Retrieved April, 2021.
10. ClinicalTrials.gov Identifier: NCT03961698. valuation of IPI-549 Combined With Front-line Treatments in Pts. With Triple-Negative Breast Cancer or Renal Cell Carcinoma (MARIO-3). Retrieved April, 2021.
11. ClinicalTrials.gov Identifier: NCT02500199. Phase I Study of Pyrotinib in Patients With HER2-positive Solid Tumors. Retrieved April, 2021.
12. ClinicalTrials.gov Identifier: NCT04690855. A Study to Evaluate Talazoparib, Radiotherapy and Atezolizumab in gBRCA 1/2 Negative Patients With PD-L1+ Metastatic Triple Negative Breast Cancer (TARA). Retrieved April, 2021.
13. Adams S, Diamond JR, Hamilton E, *et al.* Atezolizumab plus nab-paclitaxel in the treatment of metastatic triple-negative breast cancer with 2-year survival follow-up: A phase 1b clinical trial. *JAMA Oncol.* 2019;5:334–342
14. Owonikoko TK, Dahlberg SE, Sica G, *et al.* (2019). Randomized Phase II Trial of Cisplatin and Etoposide in Combination With Veliparib or Placebo for Extensive-Stage Small-Cell Lung Cancer: ECOG-ACRIN 2511 Study. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*, 37(3), 222–229. <https://doi.org/10.1200/JCO.18.00264>
15. Scalise M, Galluccio M, Console L, Pochini L, Indiveri C. (2018). The Human SLC7A5 (LAT1): The Intriguing Histidine/Large Neutral Amino Acid Transporter and Its Relevance to Human Health. *Frontiers in chemistry*, 6, 243. <https://doi.org/10.3389/fchem.2018.00243>

16. Häfliger P, Charles RP. (2019). The L-Type Amino Acid Transporter LAT1-An Emerging Target in Cancer. *International journal of molecular sciences*, 20(10), 2428. <https://doi.org/10.3390/ijms20102428>
17. Kuo LJ, Yang LX. γ -H2AX- A novel biomarker for DNA double-strand breaks. *In Vivo*. 2008. p. 305–310. PMID: 18610740
18. Lee SK, Huang S, Lu W, Lev DC, Price JE. Vascular endothelial growth factor expression promotes the growth of breast cancer brain metastases in nude mice. *Clin Exp Metastasis*. 2004;21(2):107–118. PMID: 15168728
19. McEwan PE, Bailey L, Trost D, *et al*. Percutaneous Hepatic Perfusion With Filtered Melphalan for Localized Treatment of Metastatic Hepatic Disease: A Risk Assessment. *Int J Toxicol*. 2018;37(6):434–447. PMID: 30453808
20. Altilio T, Otis-Green S. *Oxford Textbook of Palliative Medicine*. Oxford University Press, 1993. 109
21. O'Toole DM, Golden AM. Evaluating cancer patients for rehabilitation potential. *West J Med*. 1991 Oct. 155(4):384-7.
22. NIH, National Cancer Institute, & Cancer Therapy Evaluation Program. Common terminology criteria for adverse Events (CTCAE) Version 5. Retrieved April, 2021, from https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm
23. Eisenhauer EA, Therasse P, Bogaerts J, *et al*. (2009). New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *European journal of cancer (Oxford, England: 1990)*, 45(2), 228–247. <https://doi.org/10.1016/j.ejca.2008.10.026>
24. Simon, R., 1989. Optimal two-stage designs for phase II clinical trials. *Controlled clinical trials*, 10(1), pp.1-10.
25. Clopper CJ, Pearson ES. The Use of Confidence or Fiducial Limits Illustrated in the Case of the Binomial. *Biometrika*. 1934;26(4):404.
26. Bland JM, Altman DG. Statistics Notes: Survival probabilities (the Kaplan-Meier method). *BMJ*. 1998;
27. Brookmeyer R, Crowley J. A Confidence Interval for the Median Survival Time. *Biometrics*. 1982;38(1):29.
28. Rao MB, Klein JP, Moeschberger ML. Survival Analysis Techniques for Censored and Truncated Data. *Technometrics*. 1998;40(2):159.
29. Greenwood M. The errors of sampling of the survivorship tables. *Reports Public Heal Stat Subj*. 1926;33(1):26.
30. Kalbfleisch JD, Prentice RL. The Statistical Analysis of Failure Data. *IEEE Transactions on Reliability*. 1986. p. 11.
31. National Institutes of Health. "Leptomeningeal Disease." *NCI Dictionary of Cancer Terms*, National Cancer Institute, 2021, www.cancer.gov/publications/dictionaries/cancer-terms/def/leptomeningeal-metastasis.
32. Morikawa A, Jordan L, Rozner R, *et al*. (2017). Characteristics and Outcomes of Patients With Breast Cancer With Leptomeningeal Metastasis. *Clinical breast cancer*, 17(1), 23–28. <https://doi.org/10.1016/j.clbc.2016.07.002>
33. Torrejón D, Oliveira M, Cortes,J, *et al*. (2013). Implication of breast cancer phenotype for patients with leptomeningeal carcinomatosis. *Breast (Edinburgh, Scotland)*, 22(1), 19–23. <https://doi.org/10.1016/j.breast.2012.10.009>
34. Morikawa A, Jordan L, Rozner R, *et al*. (2017). Characteristics and Outcomes of Patients With Breast Cancer With Leptomeningeal Metastasis. *Clinical breast cancer*, 17(1), 23–28. <https://doi.org/10.1016/j.clbc.2016.07.002>

35. Torrejón D, Oliveira M, Cortes J, *et al.* (2013). Implication of breast cancer phenotype for patients with leptomeningeal carcinomatosis. *Breast (Edinburgh, Scotland)*, 22(1), 19–23. <https://doi.org/10.1016/j.breast.2012.10.009>
36. Lin NU, Lee EQ, Aoyama H, *et al.* Response Assessment in Neuro-Oncology (RANO) group (2015). Response assessment criteria for brain metastases: proposal from the RANO group. *The Lancet. Oncology*, 16(6), e270–e278. [https://doi.org/10.1016/S1470-2045\(15\)70057-4](https://doi.org/10.1016/S1470-2045(15)70057-4)
37. Nilapwar, S. M., Nardelli, M., Westerhoff, H. V., & Verma, M. (2011). Absorption spectroscopy. *Methods in enzymology*, 500, 59–75. <https://doi.org/10.1016/B978-0-12-385118-5.00004-9>
38. Ramsharan Singh, Jin Chen, Teresa Miller, Michael Bergren & Rangan Mallik (2016): Solution stability of Captisol-stabilized melphalan (Evomela) versus Propylene glycol-based melphalan hydrochloride injection, *Pharmaceutical Development and Technology*, DOI: 10.1080/10837450.2016.1265557
39. D'Elia-Eckbold, Jaclyn; Gwinn, Laura; Reynolds, Erin; Olkowski, Colleen; Brebner, Kim; Egan, Richard; Willis, Bradley. Discovery Tissue Distribution by Quantitative Whole-Body Autoradiography in Female Athymic Nude (Nu+/Nu+) Xenograft Mice Administered a Single Intravenous Dose of [¹⁴C]QBS10072S. *Drug Metabolism and Pharmacokinetics Report*, 1760D-1801, Quadriga BioSciences, Inc., 20 Sept. 2019.
40. Pan Z, Yang G, He H, Yuan T, Wang Y, Li Y, *et al.* Leptomeningeal metastasis from solid tumors: Clinical features and its diagnostic implication. *Sci Rep*. 2018;8:10445
41. Le Rhun E, Taillibert S, Chamberlain MC. Carcinomatous meningitis: Leptomeningeal metastases in solid tumors. *Surg Neurol Int*. 2013;4:S265-288
42. Aaronson NK, Ahmedzai S, Bergman B, *et al.* The European Organisation for Research and Treatment of Cancer QLQ-C30: A quality-of-life instrument for use in international clinical trials in oncology. *Journal of the National Cancer Institute* 1993; 85: 365-376.
43. Fayers PM, Aaronson NK, Bjordal K, Groenvold M, Curran D, Bottomley A, on behalf of the EORTC Quality of Life Group. *The EORTC QLQ-C30 Scoring Manual (3rd Edition)*. Published by: European Organisation for Research and Treatment of Cancer, Brussels 2001.
44. Taphoorn MJ, Claassens L, Aaronson NK, *et al*; on behalf of the EORTC Quality of Life Group, and Brain Cancer, NCIC and Radiotherapy Groups. An international validation study of the EORTC brain cancer module (EORTC QLQ-BN20) for assessing health-related quality of life and symptoms in brain cancer patients. *European Journal of Cancer*. 2010 Apr;46(6):1033-40. doi: 10.1016/j.ejca.2010.01.012.
45. Hu L, Ru K, Zhang L, *et al.* (2014). Fluorescence in situ hybridization (FISH): an increasingly demanded tool for biomarker research and personalized medicine. *Biomarker research*, 2(1), 3. <https://doi.org/10.1186/2050-7771-2-3>
46. Magaki S, Hojat SA, Wei B, So A, Yong WH. (2019). An Introduction to the Performance of Immunohistochemistry. *Methods in molecular biology (Clifton, N.J.)*, 1897, 289–298. https://doi.org/10.1007/978-1-4939-8935-5_25
47. Patchell RA, Tibbs PA, Regine WF, *et al.* Postoperative Radiotherapy in the Treatment of Single Metastases to the Brain: A Randomized Trial. *JAMA*. 1998;280(17):1485–1489. doi:10.1001/jama.280.17.1485
48. Hulsbergen AFC, Claes A, Kavouridis VK, Ansaripour A, Nogarede C, Hughes ME, Smith TR, Brastianos PK, Verhoeff JJC, Lin NU, Broekman MLD. Subtype switching in breast cancer brain metastases: a multicenter analysis. *Neuro Oncol*. 2020 Aug 17;22(8):1173-1181. doi: 10.1093/neuonc/noaa013. PMID: 31970416; PMCID: PMC7471502.
49. Papin-Michault C, Bonnetaud C, Dufour M, Almairac F, Coutts M, Patouraux S, Virolle T, Darcourt J, Burel-Vandenbos F. Study of LAT1 Expression in Brain Metastases: Towards a Better Understanding of the Results of Positron Emission Tomography Using Amino Acid

- Tracers. *PLoS One*. 2016 Jun 8;11(6):e0157139. doi: 10.1371/journal.pone.0157139. PMID: 27276226; PMCID: PMC4898730.
50. Ichinoe, M., Mikami, T., Yanagisawa, N., Yoshida, T., Hana, K., Endou, H., Okayasu, I., Sengoku, N., Ogata, H., Saegusa, M., Shibuya, K., & Murakumo, Y. (2021). Prognostic values of L-type amino acid transporter 1 and CD98hc expression in breast cancer. *Journal of clinical pathology*, 74(9), 589–595. <https://doi.org/10.1136/jclinpath-2020-206457>
 51. Fedchenko, N., & Reifenrath, J. (2014). Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue - a review. *Diagnostic pathology*, 9, 221. <https://doi.org/10.1186/s13000-014-0221-9>
 52. College of American Pathologists. Estrogen and Progesterone Receptor Testing in Breast Cancer Guideline Update. *College of American Pathologists with the American Society of Clinical Oncology*, 2020. <https://www.cap.org/protocols-and-guidelines/cap-guidelines/current-cap-guidelines/guideline-recommendations-for-immunohistochemical-testing-of-estrogen-and-progesterone-receptors-in-breast-cancer>
 53. ENHERTU® (fam-trastuzumab deruxtecan-nxki); *Official HCP Website*. (2023, August). www.enhertuhcp.com. <https://www.enhertuhcp.com/en>
 54. Katano, A., & Yamashita, H. (2022). Brain metastasis: Recent treatment modalities and future-perspectives (Review). *Oncology Letters*, 23(6). <https://doi.org/10.3892/ol.2022.13311>
 55. Lv Y, Ma X, Du Y, Feng J. Understanding Patterns of Brain Metastasis in Triple-Negative Breast Cancer and Exploring Potential Therapeutic Targets. *Onco Targets Ther*. 2021 Jan 22;14:589-607. doi: 10.2147/OTT.S293685. PMID: 33519208; PMCID: PMC7837592.
 56. Bhambhani HP, Granucci M, Rodrigues A, Kakusa BW, Hayden Gephart M. The primary sites leading to brain metastases: Shifting trends at a tertiary care center. *J Clin Neurosci*. 2020 Oct;80:121-124. doi: 10.1016/j.jocn.2020.08.006. Epub 2020 Aug 19. PMID: 33099333.

12. PROTOCOL HISTORY

Version Date *	Change Summary
1 24 Sept 2021	Initial submission to SCI Scientific Review Committee (SRC)
2 13 Dec 2021	Revisions to respond to FDA review comments regarding definition of TNBC, maximum allowable prior regimens, and washout for live vaccines.
3 14-Dec-2021	Revisions to respond to FDA review comments regarding reference to ASCO/CAP guidelines, added discussion about ER and PR expression levels.
4 13-Jun-2022	– Include NCT number on cover page – Removed Chirag Patel, MD from Sub-Is following cover page – Corrected references to Quadriga from industry sponsor to privately held pharmaceutical partner – Updated dose level from 12mg/m² to 18mg/m² per newly available Phase I safety data – IP manufacturing company from NerPharMa to Societal CDMO, Inc. per name change – Corrected window of IP administration +/-3 days in Cycle 3+ (to match previously approved, correct info in Schedule of Events Section 6.1) – Removed incorrect barring of nausea and vomiting prophylaxis – Updated premedication guidelines to match Stanford Medicine Neuro-Oncology standard of care – Updated vitals C1D1 to 2x for Cohort 1 and 5x for Cohort 2 – Updated PKs C1D1 to 4x for Cohort 2, clarified wording
5 27-Oct-2022 (not submitted to FDA or IRB separately)	– Revised Table 7: LMD Progression Evidence Scale to clarify distinction between item #2 and #5 – Reduced vital sign, blood, and CSF collection in Cohort 2's C1D1 from five timepoints to two – Added references throughout protocol to Imaging Manual. Moved some imaging details out of protocol to Imaging Manual to maintain data integrity but allow for potential updates to Stanford brain tumor imaging best practices without triggering a protocol up-version – Added option to step down dose of QBS72S drug to 12 mg/m² if participant is experiencing intolerable toxicity or infusion reaction despite supportive care but there is evidence of potential clinical benefit to continuing – Removed exclusion criteria #5 as it was covered adequately by inclusion #2 – PI clarified that excl #5 was intended that participants with <i>only</i> spinal or brain stem disease and no LMD or brain parenchymal disease should be excluded. PI additionally clarified that existing systemic disease burden outside of the brain, including spinal, is allowed if participants meet incl #2. – Because this clarification makes excl #5 overlap entirely with incl #2, excl #5 was removed and the exclusion criteria list was re-numbered. – Excl #5 now refers to potential participant history of severe reaction to QBS72S substances, excl #6 to contraindicated medications, etc. – Updated study completion timeline

Version Date *	Change Summary
6 29-Nov-2022	- Modified study title from “[...] Brain Metastases of Triple Negative Breast Cancer” to “[...] Brain Metastases of Breast Cancer” - Modified inclusion criteria and study descriptions from TNBC to breast cancer throughout the protocol - Added scientific rationale for expanding inclusion criteria - Modified definition of participants evaluable for the primary statistical endpoint to include high to moderate LAT1 expression - Clarified subset of participants evaluable for secondary and exploratory endpoints throughout Section 10 Statistics - Clarified which statistical subset of participants are being discussed throughout the protocol - Added enrollment total increase to 35-40 participants across all cohorts. We now expect to accrue 15-18 participants for Cohort 1 Stage 1 and additionally 10-12 participants for Cohort 1 Stage 2 for a total of 25-30 participants across the entirety of Cohort 1 - Added overview of LAT1 IHC analysis and reference to the Stanford MHG Lab Manual
7 28-Aug-2023	- Section 1.1 Study Rationale broadened scope from TNBC to all breast cancer sub-types - Section 3.1 Eligibility Criteria - o Removed reference to registration with sponsor - o Removed reference to Study Participant Log - o Changed “untreated” to “non-irradiated” qualifying metastatic lesions - o Removed positive cytology as qualifying evidence for LMD cohort - o Consolidated conmed and washout criteria - o Changed from ‘all prior therapy tox resolve to Grade 1’ to ‘reach new baseline or resolve’ - Removed remote consent – <u>former</u> Section 3.3.4 Remote Consent Process - Revised IP admin instructions for clarity per IDS and CTRU feedback Section 4.1.2 Administration - Revised dose interruption guidelines Section 5.6.2 Dose Interruptions and Delays - Rephrased Section 5.7.1 Premedication Guidelines to allow for Investigator discretion - Section 6.1 Schedule of Events - o Removed placement of Ommaya (captured in Section 3.1 Eligibility Criteria) - o Removed contraception check - o Removed archival tumor biopsy (captured in Section 8 Correlative and Special Studies) - o Telehealth now allowed for: EOT visit, safety FU visit, survival checks - o Chart review explicitly allowed for survival checks - o Safety FU visit changed from after last in-person visit to after EOT - o Added study-specific systemic imaging collection - o Added MR and systemic imaging collection timing rules - o Collecting weight optional for wheelchair users - Rewrote Section 7 Adverse Events and Reporting Procedures for clarity - Removed <u>former</u> Section 9.5 Site Documentation and Management

Version Date *	Change Summary
8 01-Nov-2023	– Added Arm 3: Enroll 10 participants with LMD from any primary cancer type ○ Changes to Protocol Synopsis, Section 1.1 Study Rationale, Section 2 Study Design, Section 3.1 Eligibility Criteria and Participant Eligibility Checklist, Section 6.1 Schedule of Events, Section 10.2.3 Exploratory Objectives, Section 10.6 Exploratory Analysis, and Section 10.7 Sample Size – Added flexibility to enrollment to Arm 2 and Arm 3 – allow five low LAT1 expressors to be replaced in each arm – Non-substantive changes in wording in Section 4.1.3 Dose, Section 5.4 Treatment Administration Instructions, and Section 5.5 Treatment Duration ○ Changes meant to remove duplicate information from the protocol and add clarity – Added info about recent cerebral edema SUSAR to Section 1.3 Potential Risks and Benefits and to Section 5.6.3 Potential Adverse Events
9 31-May-2024	– Modified inclusion criteria: ○ Added washout windows for kinase inhibitors: PARP, EGFR, ALK, NTRK, RET, HER2, and BRAF-MEK ○ corticosteroid washout wording altered from 14 days simple to 7 days with stipulations

* Latest version date should match footer date of the last protocol in this list.

13. APPENDICES

13.1. Appendix A. Revised RECIST guideline (Version 1.1)

https://ctep.cancer.gov/protocoldevelopment/docs/recist_guideline.pdf

13.2. **Appendix B. EORTC Quality of Life Questionnaires**^[42, 43, 44]

This page left intentionally blank. See following pages.



EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Please fill in your initials:

Your birthdate (Day, Month, Year):

Today's date (Day, Month, Year):

31									

	Not at All	A Little	Quite a Bit	Very Much
1. Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2. Do you have any trouble taking a <u>long</u> walk?	1	2	3	4
3. Do you have any trouble taking a <u>short</u> walk outside of the house?	1	2	3	4
4. Do you need to stay in bed or a chair during the day?	1	2	3	4
5. Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:

	Not at All	A Little	Quite a Bit	Very Much
6. Were you limited in doing either your work or other daily activities?	1	2	3	4
7. Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8. Were you short of breath?	1	2	3	4
9. Have you had pain?	1	2	3	4
10. Did you need to rest?	1	2	3	4
11. Have you had trouble sleeping?	1	2	3	4
12. Have you felt weak?	1	2	3	4
13. Have you lacked appetite?	1	2	3	4
14. Have you felt nauseated?	1	2	3	4
15. Have you vomited?	1	2	3	4
16. Have you been constipated?	1	2	3	4

Please go on to the next page

During the past week:

	Not at All	A Little	Quite a Bit	Very Much
17. Have you had diarrhea?	1	2	3	4
18. Were you tired?	1	2	3	4
19. Did pain interfere with your daily activities?	1	2	3	4
20. Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21. Did you feel tense?	1	2	3	4
22. Did you worry?	1	2	3	4
23. Did you feel irritable?	1	2	3	4
24. Did you feel depressed?	1	2	3	4
25. Have you had difficulty remembering things?	1	2	3	4
26. Has your physical condition or medical treatment interfered with your <u>family</u> life?	1	2	3	4
27. Has your physical condition or medical treatment interfered with your <u>social</u> activities?	1	2	3	4
28. Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you29. How would you rate your overall health during the past week?

1 2 3 4 5 6 7

Very poor

Excellent

30. How would you rate your overall quality of life during the past week?

1 2 3 4 5 6 7

Very poor

Excellent



EORTC QLQ - BN20

Patients sometimes report that they have the following symptoms. Please indicate the extent to which you have experienced these symptoms or problems during the past week.

During the past week:	Not at All	A Little	Quite a Bit	Very Much
31. Did you feel uncertain about the future?	1	2	3	4
32. Did you feel you had setbacks in your condition?	1	2	3	4
33. Were you concerned about disruption of family life?	1	2	3	4
34. Did you have headaches?	1	2	3	4
35. Did your outlook on the future worsen?	1	2	3	4
36. Did you have double vision?	1	2	3	4
37. Was your vision blurred?	1	2	3	4
38. Did you have difficulty reading because of your vision?	1	2	3	4
39. Did you have seizures?	1	2	3	4
40. Did you have weakness on one side of your body?	1	2	3	4
41. Did you have trouble finding the right words to express yourself?	1	2	3	4
42. Did you have difficulty speaking?	1	2	3	4
43. Did you have trouble communicating your thoughts?	1	2	3	4
44. Did you feel drowsy during the daytime?	1	2	3	4
45. Did you have trouble with your coordination?	1	2	3	4
46. Did hair loss bother you?	1	2	3	4
47. Did itching of your skin bother you?	1	2	3	4
48. Did you have weakness of both legs?	1	2	3	4
49. Did you feel unsteady on your feet?	1	2	3	4
50. Did you have trouble controlling your bladder?	1	2	3	4



EORTC QLQ-C30 (versión 3)

Estamos interesados en conocer algunas cosas sobre usted y su salud. Por favor, responda a todas las preguntas personalmente, rodeando con un círculo el número que mejor se aplique a su caso. No hay respuestas "correctas" o "incorrectas". La información que nos proporcione será estrictamente confidencial.

Por favor, escriba sus iniciales:

--	--	--	--	--

Su fecha de nacimiento (día, mes, año):

--	--	--	--	--	--	--	--	--	--

Fecha de hoy (día, mes, año):

31

--	--	--	--	--	--	--	--	--	--

		En absoluto	Un poco	Bastante	Mucho
1.	¿Tiene alguna dificultad para realizar actividades que requieren de un esfuerzo importante, como llevar una bolsa de compras pesada o una maleta?	1	2	3	4
2.	¿Tiene alguna dificultad para dar un paseo <u>largo</u> ?	1	2	3	4
3.	¿Tiene alguna dificultad para dar un paseo <u>corto</u> fuera de casa?	1	2	3	4
4.	¿Tiene que permanecer en la cama o sentado/a en una silla durante el día?	1	2	3	4
5.	¿Necesita ayuda para comer, vestirse, asearse o ir al sanitario?	1	2	3	4

Durante la semana pasada:

		En absoluto	Un poco	Bastante	Mucho
6.	¿Ha tenido algún impedimento para hacer su trabajo u otras actividades cotidianas?	1	2	3	4
7.	¿Ha tenido algún impedimento para realizar sus aficiones u otras actividades de ocio?	1	2	3	4
8.	¿Sintió que se le cortó la respiración?	1	2	3	4
9.	¿Ha tenido dolor?	1	2	3	4
10.	¿Necesitó parar para descansar?	1	2	3	4
11.	¿Ha tenido dificultades para dormir?	1	2	3	4
12.	¿Se ha sentido débil?	1	2	3	4
13.	¿Le ha faltado el apetito?	1	2	3	4
14.	¿Ha tenido náuseas?	1	2	3	4
15.	¿Ha vomitado?	1	2	3	4
16.	¿Ha estado estreñido/a?	1	2	3	4

Por favor, continúe en la página siguiente

SPANISH (MEXICO)

Durante la semana pasada:

Durante la semana pasada:		En absoluto	Un poco	Bastante	Mucho
17.	¿Ha tenido diarrea?	1	2	3	4
18.	¿Estuvo cansado/a?	1	2	3	4
19.	¿El dolor interfirió en sus actividades diarias?	1	2	3	4
20.	¿Ha tenido dificultad para concentrarse en cosas como leer el periódico o ver la televisión?	1	2	3	4
21.	¿Se sintió nervioso/a?	1	2	3	4
22.	¿Se sintió preocupado/a?	1	2	3	4
23.	¿Se sintió irritable?	1	2	3	4
24.	¿Se sintió deprimido/a?	1	2	3	4
25.	¿Ha tenido dificultades para recordar cosas?	1	2	3	4
26.	¿Su estado físico o el tratamiento médico han interferido en su vida <u>familiar</u> ?	1	2	3	4
27.	¿Su estado físico o el tratamiento médico han interferido en sus actividades <u>sociales</u> ?	1	2	3	4
28.	¿Su estado físico o el tratamiento médico le han causado dificultades financieras?	1	2	3	4

En las siguientes preguntas por favor, dibuje un círculo en el número del 1 al 7 que mejor se aplique a usted:

29. ¿Cómo valoraría, en general, su salud durante la semana pasada?

1	2	3	4	5	6	7
Pésima						Excelente

30. ¿Cómo valoraría, en general, su calidad de vida durante la semana pasada?

1	2	3	4	5	6	7
Pésima						Excelente



EORTC QLQ - BN20

Los pacientes a veces dicen que tienen los siguientes síntomas. Por favor indique hasta qué punto ha experimentado usted estos síntomas o problemas durante la semana pasada.

Durante la semana pasada:	Para nada	Un poco	Bastante	Mucho
31. ¿Se sintió desconcertado/a acerca del futuro?	1	2	3	4
32. ¿Sintió que hubo un empeoramiento en su estado?	1	2	3	4
33. ¿Estuvo preocupado/a por las alteraciones en la vida familiar?	1	2	3	4
34. ¿Tuvo dolores de cabeza?	1	2	3	4
35. ¿Empeoraron sus perspectivas de futuro?	1	2	3	4
36. ¿Tuvo visión doble?	1	2	3	4
37. ¿Tuvo visión borrosa?	1	2	3	4
38. ¿Tuvo dificultades al leer?	1	2	3	4
39. ¿Tuvo convulsiones?	1	2	3	4
40. ¿Tuvo debilidad en uno de los lados de su cuerpo?	1	2	3	4
41. ¿Tuvo problemas en encontrar las palabras adecuadas para expresarse?	1	2	3	4
42. ¿Tuvo dificultades al hablar?	1	2	3	4
43. ¿Tuvo problemas al comunicar sus pensamientos?	1	2	3	4
44. ¿Se sintió soñoliento/a durante el día?	1	2	3	4
45. ¿Tuvo problemas con su coordinación?	1	2	3	4
46. ¿Le molestó perder cabello?	1	2	3	4
47. ¿Le molestó la comezón en la piel?	1	2	3	4
48. ¿Tuvo debilidad en ambas piernas?	1	2	3	4
49. ¿Se sintió inestable estando de pie?	1	2	3	4
50. ¿Tuvo problemas controlando su vejiga?	1	2	3	4

All rights reserved. No part of this manual covered by copyrights hereon may be reproduced or transmitted in any form or by any means without prior permission of the copyright holder.

The EORTC QLQ-C30 (in all versions), and the modules which supplement it, are copyrighted and may not be used without prior written consent of the EORTC Data Center.

Requests for permission to use the EORTC QLQ-C30 and the modules, or to reproduce or quote materials contained in this manual, should be addressed to:

QL Coordinator
Quality of Life Unit,
EORTC Data Center,
Avenue E Mounier 83 - B11,
1200 Brussels,
BELGIUM

Tel: +32 2 774 1611
Fax: +32 2 779 4568
Email: abo@eortc.be

Copyright © 1995, 1999, 2001 EORTC, Brussels.
D/2001/6136/001
ISBN 2-9300 64-22-6

Third edition, 2001

EORTC QLQ-C30 Scoring Manual

The EORTC QLQ-C30

Introduction

The EORTC quality of life questionnaire (QLQ) is an integrated system for assessing the health-related quality of life (QoL) of cancer patients participating in international clinical trials. The core questionnaire, the QLQ-C30, is the product of more than a decade of collaborative research. Following its general release in 1993, the QLQ-C30 has been used in a wide range of cancer clinical trials, by a large number of research groups; it has additionally been used in various other, non-trial studies.

This manual contains scoring procedures for the QLQ-C30 versions 1.0, (+3), 2.0 and 3.0; it also contains summary information about supplementary modules.

All publications relating to the QLQ should use the scoring procedures described in this manual.

This manual will be updated at regular intervals, to reflect future changes to the QLQ and to incorporate new supplementary modules.

General principles of scoring

The QLQ-C30 is composed of both multi-item scales and single-item measures. These include five functional scales, three symptom scales, a global health status / QoL scale, and six single items. Each of the multi-item scales includes a different set of items - no item occurs in more than one scale.

All of the scales and single-item measures range in score from 0 to 100. A high scale score represents a higher response level.

Thus a **high score for a functional scale** represents a *high / healthy level of functioning*,
a **high score for the global health status / QoL** represents a *high QoL*,
but a **high score for a symptom scale / item** represents a *high level of symptomatology / problems*.

The principle for scoring these scales is the same in all cases:

1. Estimate the average of the items that contribute to the scale; this is the *raw score*.
2. Use a linear transformation to standardise the raw score, so that scores range from 0 to 100; a higher score represents a higher ("better") level of functioning, or a higher ("worse") level of symptoms.

Coding of the scoring procedure is presented in Appendix 3 for three major statistical packages.

Technical Summary

In practical terms, if items $I_1, I_2, \dots, I_n$ are included in a scale, the procedure is as follows:

Raw score

Calculate the raw score

$$\text{RawScore} = RS = (I_1 + I_2 + \dots + I_n) / n$$

Linear transformation

Apply the linear transformation to 0-100 to obtain the score S .

$$\text{Functional scales:} \quad S = \left\{ 1 - \frac{(RS - 1)}{\text{range}} \right\} \times 100$$

$$\text{Symptom scales / items:} \quad S = \{(RS - 1) / \text{range}\} \times 100$$

$$\text{Global health status / QoL:} \quad S = \{(RS - 1) / \text{range}\} \times 100$$

Range is the difference between the maximum possible value of RS and the minimum possible value. The QLQ-C30 has been designed so that all items in any scale take the same range of values. Therefore, the range of RS equals the range of the item values. Most items are scored 1 to 4, giving $\text{range} = 3$. The exceptions are the items contributing to the global health status / QoL, which are 7-point questions with $\text{range} = 6$, and the initial yes/no items on the earlier versions of the QLQ-C30 which have $\text{range} = 1$.

Scoring the EORTC QLQ-C30 version 3.0

Table 1: Scoring the QLQ-C30 version 3.0

	Scale	Number of items	Item range*	Version 3.0 Item numbers	Function scales
Global health status / QoL					
Global health status/QoL (revised) [†]	QL2	2	6	29, 30	
Functional scales					
Physical functioning (revised) [†]	PF2	5	3	1 to 5	F
Role functioning (revised) [†]	RF2	2	3	6, 7	F
Emotional functioning	EF	4	3	21 to 24	F
Cognitive functioning	CF	2	3	20, 25	F
Social functioning	SF	2	3	26, 27	F
Symptom scales / items					
Fatigue	FA	3	3	10, 12, 18	
Nausea and vomiting	NV	2	3	14, 15	
Pain	PA	2	3	9, 19	
Dyspnoea	DY	1	3	8	
Insomnia	SL	1	3	11	
Appetite loss	AP	1	3	13	
Constipation	CO	1	3	16	
Diarrhoea	DI	1	3	17	
Financial difficulties	FI	1	3	28	

* Item range is the difference between the possible maximum and the minimum response to individual items; most items take values from 1 to 4, giving range = 3.

† (revised) scales are those that have been changed since version 1.0, and their short names are indicated in this manual by a suffix "2" – for example, PF2.

For all scales, the *RawScore*, *RS*, is the mean of the component items:

$$\text{RawScore} = RS = (I_1 + I_2 + \dots + I_n) / n$$

Then for **Functional scales**:

$$\text{Score} = \left\{ 1 - \frac{(RS - 1)}{\text{range}} \right\} \times 100$$

and for **Symptom scales / items and Global health status / QoL**:

$$\text{Score} = \left\{ (RS - 1) / \text{range} \right\} \times 100$$

Examples:

Emotional functioning

$$\begin{aligned} \text{RawScore} &= (Q_{21} + Q_{22} + Q_{23} + Q_{24}) / 4 \\ \text{EF Score} &= \left\{ 1 - (\text{RawScore} - 1) / 3 \right\} \times 100 \end{aligned}$$

Fatigue

$$\begin{aligned} \text{RawScore} &= (Q_{10} + Q_{12} + Q_{18}) / 3 \\ \text{FA Score} &= \left\{ (\text{RawScore} - 1) / 3 \right\} \times 100 \end{aligned}$$



EORTC QLQ-BN20 Scoring Manual

The Brain Cancer module is a supplementary questionnaire module to be employed in conjunction with the QLQ-C30. The QLQ-BN20 incorporates four multi-item scales to assess uncertainty about the future, visual disorder, motor dysfunction and communication deficit. In addition, seven single items assess headaches, seizures, drowsiness, hair loss, itchy skin, weakness of legs, and bladder control.

The scoring approach for the QLQ-BN20 is identical in principle to that for the symptom scales and single items of the QLQ-C30. All scoring information specific to the QLQ-BN20 is presented in Table 1.

Interpretation:

All of the scales and single-item measures range in score from 0 to 100. A high score for the scales and single items represents a high level of symptomatology or problems.

Table 1. Scoring the QLQ-BN20

	Scale	Number of items (n)	Item range*	QLQ-BN20 item numbers ($I_1, I_2, \dots, I_n$)
Multi-item scales / single items				
Future uncertainty	FU	4	3	31 – 33, 35
Headaches	H	1	3	34
Visual disorder	VD	3	3	36 – 38
Seizures	S	1	3	39
Motor dysfunction	MD	3	3	40, 45, 49
Communication deficit	CD	3	3	41 – 43
Drowsiness	Dr	1	3	44
Hair loss	HL	1	3	46
Itchy skin	IS	1	3	47
Weakness of legs	WL	1	3	48
Bladder control	BC	1	3	50

* "Item range" is the difference between the possible maximum and the minimum response to individual items. All items are scored 1 to 4, giving range = 3.

Principle for scoring

1) Raw score

For each multi-item scale, calculate the average of the corresponding items.

$$\text{Raw Score} = RS = \left\{ \frac{(I_1 + I_2 + \dots + I_n)}{n} \right\}$$

For each single-item measure, the score of the concerning item corresponds to the raw score.

There are no reverse scoring items.

2) Linear Transformation

To obtain the Score S , standardize the raw score to a 0 – 100 range following the transformation:

$$S = \left\{ \frac{(RS-1)}{range} \right\} \times 100$$

For directions on Missing Data or for more detailed information on the Interpretation of Scores, we redirect to the EORTC QLQ-C30 Scoring Manual (2001).

Reference

Taphoorn MJ, Claassens L, Aaronson NK, Coens C, Mauer M, Osoba D, Stupp R, Mirimanoff RO, van den Bent MJ, Bottomley A; on behalf of the EORTC Quality of Life Group, and Brain Cancer, NCIC and Radiotherapy Groups. An international validation study of the EORTC brain cancer module (EORTC QLQ-BN20) for assessing health-related quality of life and symptoms in brain cancer patients. *European Journal of Cancer*. 2010 Apr;46(6):1033-40. doi: 10.1016/j.ejca.2010.01.012.

Further questions or remarks regarding the scoring algorithms for the QLQ-BN20 can be directed to the QOL Specialist at the Quality of Life Department of the EORTC.