Abbreviated Title: Phase II Study LB100 gliomas

NIH Protocol #: 17C0037

Title: Phase II Trial of LB100, a Protein Phosphatase 2A Inhibitor, in Recurrent Gliomas

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NCT Number: NCT03027388

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Investigational Agents:

Drug Name:	LB100
IND Number:	132947
Sponsor:	Center for Cancer Research, National Cancer Institute
Manufacturer:	Lixte Biotechnology Holdings, Inc.
Supplier:	Lixte Biotechnology Holdings, Inc.

PRÉCIS

Background:

- Primary gliomas are an incurable disease in spite of aggressive multimodality therapy consisting of craniotomy, irradiation, and chemotherapy.(1) Therapeutic options for patients with recurrent glioma are limited, and there is an unmet need to identify more effective agents.
- LB100, a water soluble small molecule novel protein phosphatase 2A (PP2A) inhibitor, was commercially developed through a CRADA based on our previous intramural research.(2) This compound has shown to be effective in a variety of cancer types in both in vitro and in vivo models. Preclinical studies indicate LB100 has in vitro and in vivo activity as a single agent as well as potentiating the effect of cytotoxic agents including temozolomide, docetaxel, doxorubicin, and ionizing radiation.(3-6) LB100 is active in combination with temozolomide or doxorubicin against xenografts of glioblastoma, neuroblastoma, pheochromocytoma, breast cancer, fibrosarcoma, and melanoma.(5, 7-9)
- A complete phase I study of LB100 has established its safety and the recommended Phase II dose (2.33 mg/m^2 daily for three days every 3 weeks).
- Although it is a polar compound, rodent studies suggest LB100 has activity in the brain.(10)
- Whether LB100 can across the human blood brain barrier (BBB), and at what concentration relative to the plasma level is not known. Characterizing these parameters is important because: 1) Our ongoing in vitro studies indicate that LB100 has distinct mechanisms of action at different drug concentrations (e.g. nM versus uM); 2) There are other brain tumors lacking effective medical therapies but without a BBB. Characterizing the LB100 BBB penetration profile will assist in defining its optimal clinical indication.

Objective:

To determine the pharmacokinetic (PK) properties of LB100 in glioma tumor tissues.

Eligibility:

- Patients with histologically proven glioblastoma and grades II-III astrocytomas and oligodendrogliomas.
- A clear clinical indication for another surgical resection must be present.
- Subjects must be ≥ 18 years old.
- Karnofsky performance status of ≥ 60%.
- Patients must have adequate organ function.

Design:

- This is a two stage Phase II, open label, single institution study to determine the PK and PD profile of LB100.
- The dose (established from a Phase I study) will be 2.33 mg/m^2 delivered intravenously over 2 hours.
- PK and PD effect of LB100 treated tissues will only be evaluated with pathologic confirmation of recurrent tumor. Resected material demonstrating chemoradiation treatment effect or inflammatory response will not be included in the analysis.
- PK will be determined by quantitating LB100 in tumor tissues removed at various time points.
- The primary endpoint is PK response, defined as a binary variable indicating the presence/absence of LB100 in tumor tissues.
- PD effect is defined as statistically significant elevation of phospho-proteins in treated tumor tissues compared to untreated glioma specimens. Untreated inter-patient baseline variance and standard deviation (SD) will be calculated. Post-treatment PD effect difference greater than 2.5 times the baseline SD is statistically significant at the .05 significance level. Due to relatively small sample size, t-distribution is to be used to calculate the cutoff defining the PD response.
- Up to 25 patients may be enrolled to obtain 8 evaluable subjects. A two-stage design will be used. Five patients will be initially treated. If at least one of five demonstrates PK activity, 3 additional subjects will be enrolled. PK effect will be declared to be significant if at least 2 of the 8 patients demonstrate a PK response (presence of LB100 in tumor tissue).

TABLE OF CONTENTS

STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1. INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

To determine the pharmacokinetic (PK) properties of LB100 in gliomas tumor tissues

1.1.2 Secondary Objectives

1.1.2.1 To determine the concentration LB100 and its major metabolite, 7-oxabicyclo [2.2.1] heptanes-2,3-dicarboxylic acid (LB100M) in glioma tumor tissue when a known non-toxic dose of LB100 is delivered intravenously over 2 hours.

1.1.2.2 To determine the PD effect of LB100 in glioma tumor tissue.

1.1.2.3 To determine the plasma concentration and calculated PK parameters of LB100 and LB100M (endothall).

1.1.2.4 To determine changes in phospho-protein expression in circulating PBMC.

1.1.2.5 Intra-patient PD effect in PBMC and tumor tissue will be evaluated in all subjects for presence of correlation to identify potential predictive markers.

1.2 BACKGROUND AND RATIONALE

1.2.1 Glioblastoma

Glioblastoma (GB) is a primary brain tumor and the most common type of gliomas. Although the incidence rate of 3 per 100,000 is relatively low compared to other cancer types, this disease accounts for disproportionately high cancer associated morbidity and mortality due to involvement of the central nervous system and lack of effective therapies(11). The average overall survival of treated GB patients ranges from 8 to 14 months with only 9.8% alive 5 years after diagnosis(12, 13); these are figures that have not significantly changed over the past decades in spite of improvements witnessed in surgical and imaging techniques and considerable positive strides made in treatment of other cancers. Median survival after tissue diagnosis without additional treatment is 3 months.

Optimal upfront treatment of GB consists of craniotomy to remove as much tumor tissue as possible, followed by concurrent temozolomide chemotherapy and ionizing radiation. Even for patients exposed to multimodality therapy of craniotomy, chemotherapy, and irradiation, disease recurrence is inevitable; at such time, patients face very limited therapeutic options. This dismal outlook for GB underscores the importance of identifying more effective treatment strategies. Often cited reasons for the refractory nature of GB include: 1) Intrinsic relative resistance of tumors cells to chemotherapy and irradiation; 2) Infiltrating growth pattern of tumor cells into surrounding normal brain that has two clinical consequences: a) precludes surgical removal of all tumor cells; b) tumor cells distant from the surgical margin are spared from exposure to cytotoxic dose of irradiation because radiation to the whole brain results in unacceptable toxicity; 3) Presence of a “protective” blood-brain-barrier (BBB) that prevents efficient entry of exogenously administered polar drugs into the tumor bed. It should be noted that although GB is the most common of the primary brain tumors called gliomas, other gliomas exist in the brain,

and as such are also protected blood brain barrier. The other gliomas of interest include grades II-III astrocytomas and oligodendrogliomas. These tumors are treated using the same modalities as GB and unfortunately are also refractory to treatment with limited options at recurrence. Since morphological differences exist in the BBB between these tumors which may possibly alter drug penetrance, the study will include these tumors in the analysis.

Formulation of more effective treatment strategies will require addressing these barriers.

1.2.2 Agent to be Studied, LB100

Protein phosphatase 2A (PP2A) is a ubiquitous heterotrimeric serine/threonine phosphatase involved in broad cellular functions such as signal transduction, cell differentiation, regulation of cell cycle, and DNA repair(14). Naturally occurring small molecule inhibitors of PP2A such as cantharidin and okadaic acid have drawn considerable interest in preclinical stages due to potent anti-cancer properties. However clinical utility has been limited by high toxicity. LB100 (3-[4methylpiperazine-carbonyl]-7-oxalobicyclo [2.2.1] heptane-2-carboxylic acid; NSC D753810) is a small polar molecule (MW 268), which inhibits PP2A about 80 fold more efficiently than protein phosphatase 1 (PP1)(8). This compound was commercially synthesized to specifically inhibit PP2A(15). LB100 has single agent activity in vitro and in vivo and potentiates the activity of cytotoxic agents and irradiation in vivo. The mechanism of potentiation is believed to involve modulation of cell cycle and mitotic checkpoints induced by nonspecific DNA damaging agents, facilitating cancer cells to continue in mitosis despite acute DNA damage(5, 8). LB100 also appears to affect the vasculature inducing transient vessel "leakiness" at higher doses(16).

1.2.3 LB100 Preclinical Studies

A series of preclinical studies support the potential clinical utility of LB100 in various malignancies. In addition to demonstrating single agent activity, LB100 potentiates the activity of cytotoxic agents and ionizing radiation in animal models.

Pancreatic cancer: Two cell lines exposed to LB100 demonstrated aberrant activation of CDK1, attenuation of radiation-induced Rad51 focus formation and subsequent loss of homologous recombination repair activity; these effects were not seen in normal small intestine cells. In a mouse xenograft model, LB100 resulted in significant radiosensitization with minimal weight loss(4).

Ovarian cancer: In vitro and in vivo (intraperitoneal metastatic mouse model) studies showed LB100 to sensitize ovarian carcinoma lines to cisplatin-mediated cell death. The mechanism of action was determined to be loss of cisplatin-induced cell cycle arrest(7).

Medulloblastoma: LB100 enhanced the cisplatin-mediated cytotoxic effects in vitro and in vivo(16).

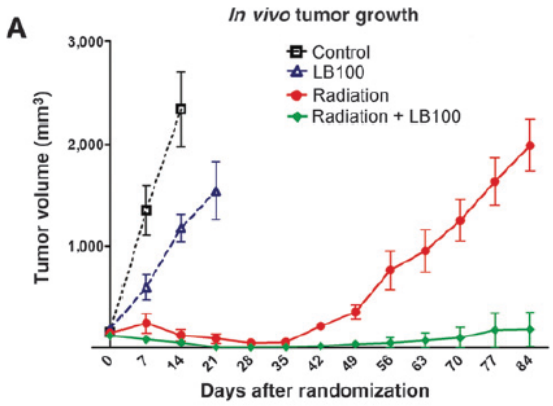
Nasopharyngeal carcinoma: Combination of LB100 with radiation treatment led to significant regression of xenografted tumor cells compared to radiation or LB100 alone. Investigators observed reduction in p53 expression along with impaired DNA repair functions(6).

Osteosarcoma: LB100 prevented repair of cisplatin-induced DNA damage in both in vitro and in vivo models leading to mitotic catastrophe and tumor cell death(9).

GB (with chemotherapy): LB1.2 (earlier generation of LB100) abrogated cell cycle arrest and enhanced the cytotoxic effects of temozolomide and doxorubicin in both in vitro and in vivo models of neuroblastoma and GB(15).

GB (with irradiation): LB100 in combination with irradiation increased expression of γ-H2AX indicative of DNA double-strand breaks, decreased p53 expression, and delayed tumor growth in a subcutaneous in vivo model of GB (**Figure 1**)(5).

Figure 1



Additional preclinical studies performed in our laboratory further supports the use of LB100 as a single agent and in combination with ionizing radiation. Meningioma is the most common primary brain tumor without effective medical therapy. Current treatment options consist only of surgery followed irradiation. We investigated three malignant meningioma cell lines (IOMM-LEE, GAR, CH-157) and found that LB100 alone at clinically achievable concentrations (5-6 μM) can exhibit antitumor effect (**Figure 2**). Cells were treated once with LB100 and viability assessed 48 hours later; x-axis represents LB100 drug concentration in uM. As seen with pancreatic and GB cells, LB100 in combination with irradiation increased γ-H2AX foci (**Figure 3**).

Figure 2

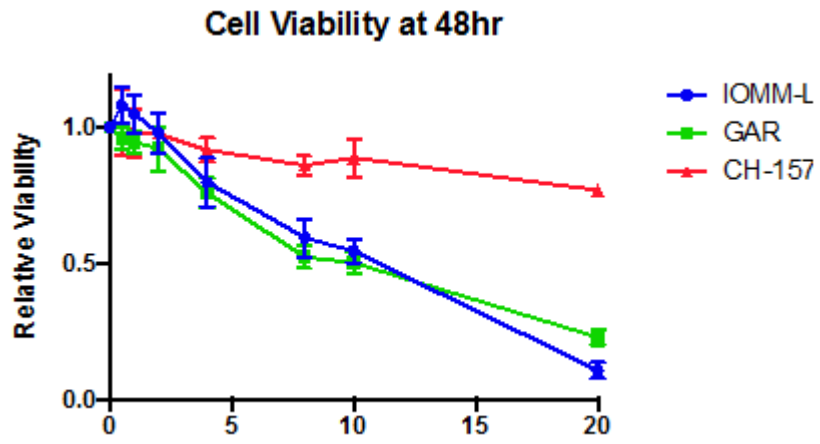
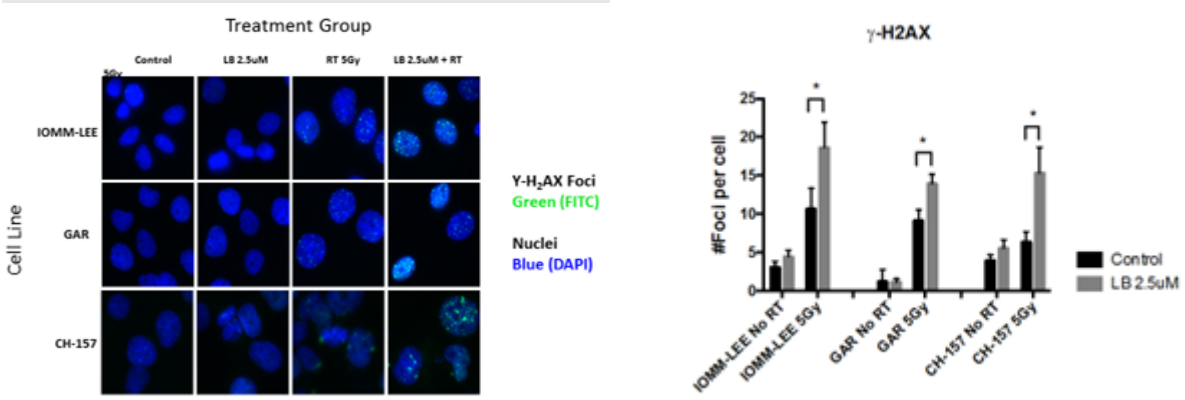


Figure 3



1.2.4 LB100 Phase I Study

A phase I, open label, single center, two-part, dose-escalation study of LB100 administered intravenously in patients with advanced solid tumors was conducted at City of Hope National Medical Center. The objectives of the study were to determine the: 1) Safety and tolerability; 2) Maximum tolerated dose (MTD); 3) Pharmacokinetics (PK). Study reached its accrual goal and closed to further patient enrollment on 5/20/2016. Dose limiting toxicity (DLT) was reversible decrease in creatinine clearance at the maximum administered dose of 3.1 mg/m^2; no other toxicities were reported. Additional patients were enrolled at a lower dose of 2.33 mg/m^2. This dose was well tolerated without creatinine elevation, and determined to be the recommended

phase II dose. On-going PK evaluation on the first cohort of subjects suggest a LB100 half-life of ~1.3 hours.

1.2.5 Rationale for a Phase II Study of LB100 in Gliomas

Preclinical studies performed in a variety of different cancer models including GB suggest that LB100 may possess anti-cancer properties as a single agent, and also potentiate cytotoxic effects of chemotherapy and ionizing radiation. However, given that LB100 is a polar molecule, a limitation of the two completed GB in vivo animal studies was the use of a subcutaneous rather than an orthotopic intracranial model. The intracranial model more closely recapitulates the native environmental conditions of human disease, including presence of at least a partial BBB. How efficiently, and to what extent LB100 is able to cross an intact BBB is unclear, although there are suggestions that LB100 can do this to a certain degree in rodents. First, administration of LB100 was shown to significantly decrease PP2A enzymatic activity in the lateral habenula (region of brain with an intact BBB) of mice. Second, we established intracranial GB models in mice. Animals were treated with one-time administration of LB100 at 1.5 mg/kg, a dose slightly higher than the human MTD; human MTD dose roughly translates to plasma concentration of 4-6 μM and this animal dose translates to a similar range (6-8 μM). Animals were sacrificed 3 hours after treatment. Liver, intracranial tumor, normal brain (animals without tumor implants) were collected and assayed by Dr. William Figg's Laboratory for presence of LB100 in these tissues. Low levels of LB100 were detected in the normal brain and liver from 2 of 3 treated animals and from the implanted tumor in 1 of 3 animals. These are encouraging results considering that the plasma half-life of LB100 in human is approximately 1.3 hours, it is therefore possible that a higher drug concentration may be detected at an earlier time point depending on exact PK parameters of LB100. Nevertheless, all of these studies were performed in rodent brains and how this may translate to human is not known.

Whether LB100 can cross the human BBB and at what concentration relative to the plasma level is not known. Even if LB100 can penetrate the human BBB, it is critically important to elucidate the specific LB100 PK properties of the brain compartment and determine pharmacodynamic (PD) effects of LB100 on glioma tissue to confirm target modulation. Even a negative study showing that LB100 is incapable of crossing the BBB is important in order to establish its clinical utility. This is because there are other brain tumors lacking effective medical therapies but without an intact BBB, where LB100 may potentially play a therapeutic role. Therefore, defining the LB100 BBB penetration profile will assist in defining its optimal clinical indication.

Additionally, morphologic differences exist in the BBB between grades of glioma that may potentially affect the penetrance of LB100. Therefore, it would be important to determine the PK/PD in these histological types in order to assess the possible therapeutic value of LB100 in a broader range of central nervous system tumors.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have histologically confirmed glioblastoma/gliosarcoma, grades II-III astrocytoma and oligodendroglioma .

2.1.1.2 Patients must have recurrent disease for which there is a clinical indication for resection.

2.1.1.3 Age ≥18 years.

2.1.1.4 Karnofsky ≥60%, see 16.1.

2.1.1.5 Patients must have adequate bone marrow function (WBC ≥ 3,000/uL ANC ≥ 1,500/mm^3, platelet count of ≥ 100,000/mm^3, and hemoglobin ≥ 10 gm/dL), adequate liver function (SGOT and bilirubin < 2 times ULN), These tests must be performed within 28 days prior to receiving drug. Eligibility level for hemoglobin may be reached by transfusion.

2.1.1.6 Patients must have a serum creatinine of ≤1.7 mg/dL. If the serum creatinine is greater than 1.7 mg/dl, a 24-hour urine creatinine clearance will be obtained and if the result of this study is within normal limits*, the patient would be eligible to enroll onto study. This test must be performed within 28 days prior to receiving drug. (*Normal Creatinine Clearance Range: Male: 90 – 130 ml/min; Female: 80 – 125 ml/min)

2.1.1.7 Patients must be in adequate general medical health to safely tolerate a craniotomy.

2.1.1.8 At the time of registration, all subjects must be removed ≥ 28 days from any investigational agents.

2.1.1.9 The effects of LB100 on the developing human fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.

2.1.1.10 Ability of subject to understand and the willingness to sign a written informed consent document indicating that they are aware of the investigational nature of this study, and that this is not a therapeutic clinical trial.

2.1.2 Exclusion Criteria

2.1.2.1 Patients who are receiving any other investigational agents.

2.1.2.2 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac

arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

2.1.2.3 Patients unwilling to undergo craniotomy.

2.1.2.4 Pregnant women are excluded from this study because the safety of PP2A inhibition on a developing fetus has not been established. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with LB100, breastfeeding should be discontinued if the mother is treated with LB100.

2.1.2.5 Patients may not have had prior chemotherapy or biologic therapy in the 4 weeks prior to study entry. For patients who have been treated with targeted therapy, 5 half-lives of that therapy (or 28 days, whichever is shorter) must have passed prior to enrollment in the study.

2.1.2.6 Known HIV-positive patients on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with LB100. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when indicated.

2.1.2.7 Patients who are receiving strong CYP450 inducers or inhibitors are ineligible

2.1.3 Recruitment Strategies

Patients with recurrent disease will be identified by the Neuro-Oncology Branch, Clinical Center. This study will be posted on NIH websites and on NIH Social media forums.

2.2 SCREENING EVALUATION

2.2.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes
- Patients will be evaluated by the Neurosurgical Team (Surgical Neurology Branch, NINDS) prior to enrollment. Patients must be in adequate health to tolerate a major operation (craniotomy). The evaluation will consist of a discussion with the patient and the neurosurgical team will review the imaging and will discuss with the patient.

Documentation/ confirmation of tumor diagnosis is required prior to enrollment. Slides from the most recent pre-registration biopsy or resection will be requested for review if the previous surgery was not performed at NIH.

A waiver of consent for these activities has been requested in section **12.5.1.**

2.2.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the study consent OR the consent for study 01-C-0129 (provided the procedure is permitted on that study) on which screening activities may also be performed. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a patient has signed the consent.

Within 28 days prior to receiving study drug the following assessments are required:

- A complete history and neurological examination (to include documentation of the patients' Karnofsky Performance Status).
- Neuro-imaging confirming tumor progression shall be performed on all patients. MRI is preferred over CT scan.
- 12-lead EKG
- Laboratory Evaluation:
    - CBC with differential, platelets, PT, PTT, INR
    - Total protein, albumin, calcium, phosphorus, magnesium, glucose, BUN, creatinine, sodium, potassium, alkaline phosphatase, ALT, AST, total bilirubin, direct bilirubin), LDH and uric acid
    - 24-hour urine collection for Creatinine Clearance (if required per section **2.1.1.6**)
    - Urine or serum β-HCG

2.3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g. when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found at https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825

2.3.1 Screen Failures

Screen failures are defined as participants who consent to participate in the clinical trial but are not subsequently assigned to the study intervention or entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this trial (screen failure) because of a potentially reversable conditions (i.e., lab abnormality) may be rescreened..

2.3.2 Treatment Assignment Procedures

Cohorts

Number	Name	Description
1	Phase 2	Phase 2 subjects

Arms

Number	Name	Description
1	LB100	Treatment with LB100

Stratifications

Name	Distinct Options	Notes
None		

Arm Assignment:

Patients in cohort 1 will be directly assigned to arm 1.

2.4 BASELINE EVALUATION

All baseline evaluation procedures should be done prior to the study treatments. They do not need to be repeated if performed within 28 days prior to study drug initiation.

- A complete history and physical (including baseline blood pressure measurement) and neurological examination (include documentation of the patients' height, weight, and Karnofsky Performance Status).
- A baseline standard NOB Gd-DPTA enhanced MRI should be performed.

3 STUDY IMPLEMENTATION

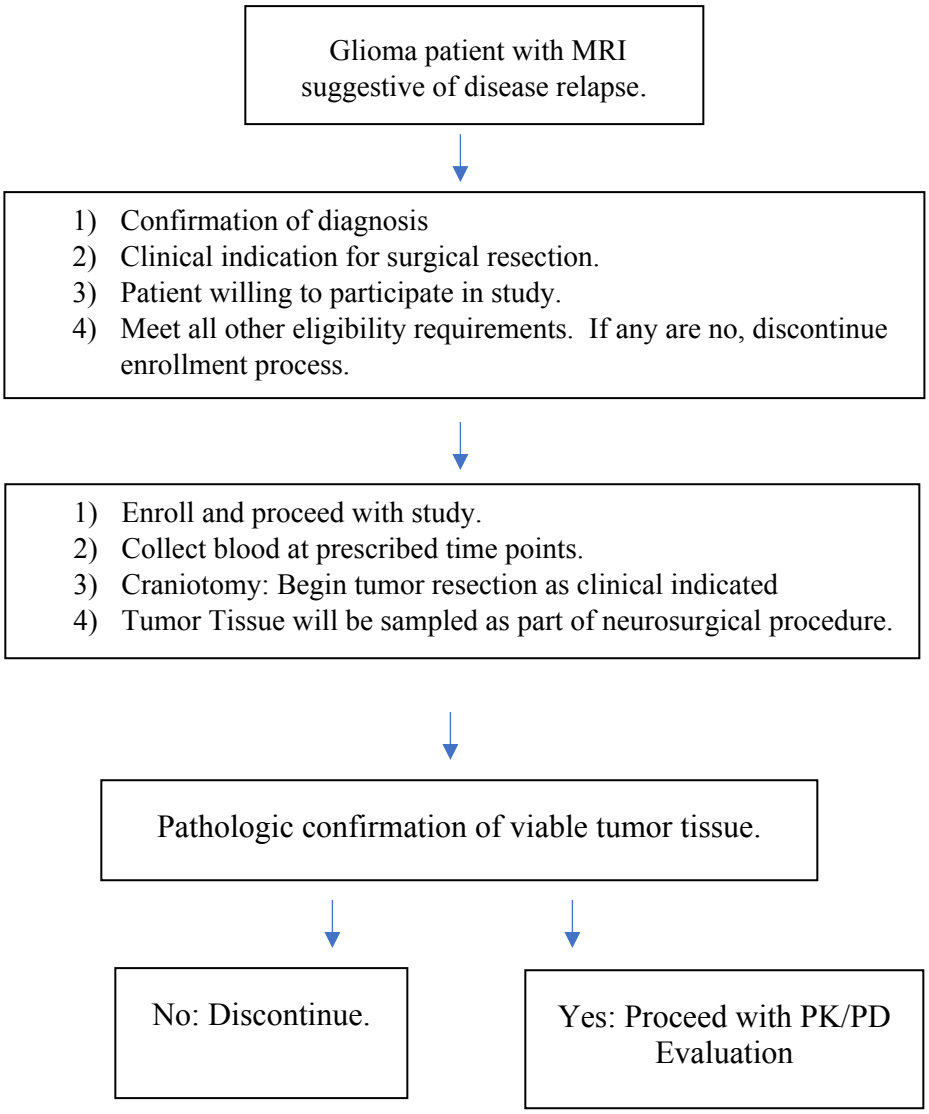
3.1 STUDY DESIGN

This is a two stage Phase II, open label, single institution study to determine the PK and PD profile of LB100 in the setting of gliomas. Patients who are planned for craniotomy will be enrolled if they meet the inclusion criteria. Patients will be admitted to the Clinical Center for the surgery. Tumor tissue will be sampled as part of neurosurgical procedure. LB-100 will be infused over 2 hours (+/- 15 minutes) via IV infusion 2 to 4 hours before surgery. The dose established from a Phase I study will be 2.33 mg/m^2. Because the tumor resection phase of the craniotomy for

recurrent gliomas typically lasts from one to 5 hours, tumor tissue will be sampled at multiple time points as clinically indicated. The exact time points of tissue removal from LB100 infusion will be recorded by a member of the study team in the OR. Plasma sampling for PK measurements of LB100 will be performed on all participating subjects. (See section **5.1**).

Up to 25 patients may be enrolled to obtain 8 evaluable patients. Five patients will be initially treated. If one of five demonstrates a PK response, 3 additional subjects will be enrolled. PK effect will be declared to be significant if at least 2 of the 8 patients demonstrate a PK response (Presence of LB100 in tumor tissue). Participants whose pathology demonstrates treatment effect or not active tumor will be replaced.

3.1.1 Design/Schema



3.2 DRUG ADMINISTRATION

LB-100 will be infused over 2 hours (+/- 15 minutes) via IV infusion. The dose established from a Phase I study will be 2.33 mg/m^2. At the end of infusion, each subject will have an EKG (within 30 minutes of the end of infusion).

3.3 FOLLOW-UP

All patients will be evaluated in person 1-3 days following craniotomy and followed for at least 30 days after infusion of LB-100 for toxicity assessment. During the first week after infusion with LB-100, renal function tests and liver function tests will be performed. Two to 3 weeks following discharge from the hospital, all patients will return to the clinic or be seen by their local physician for a physical exam and bloodwork. Given that the methodologies utilized are similar across all laboratories, no significant variability is expected and there is no anticipation that study data will be affected. Patients will be contacted by phone (or seen in clinic) at approximately 30 days post craniotomy and will be asked a non-leading question to determine whether any new AEs have occurred and to follow up on any ongoing AEs.

3.4 STUDY CALENDAR

STUDIES TO BE OBTAINED	PRE-TREATMENT	AT CRANIOTOMY	3 DAY FOLLOW UP (+/- 2 Days)	2-3 WEEK FOLLOW UP	30 DAY FOLLOW- UP
Informed Consent	X				
Medical History	X				
Physical Examination	X			X	
Tumor Imaging -MRI (preferred) or CT scan	X				
Documentation of tumor diagnosis	X				
Laboratory Evaluation[1]	X		X	X	
24 hour Creatinine clearance (if required per section **2.1.1.6**)	X				
12-lead EKG	X	X[5]			
LB100	X				
PK/PD[2, 3]	X	X			

STUDIES TO BE OBTAINED	PRE-TREATMENT	AT CRANIOTOMY	3 DAY FOLLOW UP (+/- 2 Days)	2-3 WEEK FOLLOW UP	30 DAY FOLLOW- UP
Adverse Events Notation		X	X	X	X[4]

Pre-treatment studies should be performed within 28 days of receiving study drug.

[1] Laboratory Evaluation: CBC, differential, platelets, PT, PTT, INR, total protein, albumin, calcium, phosphorus, magnesium, glucose, BUN, creatinine, sodium, potassium, uric acid, total bilirubin, alkaline phosphatase, LDH, ALT, AST. The pregnancy test for women of child bearing age will be done at screening only.

[2] Plasma PK: see section **5.1.1.1**

[3] Tissue PK: see section **5.1.1.2**

[4] Follow- up: see section **3.3**

[5]EKG within 30 minutes of the end of infusion.

3.5 SURGICAL GUIDELINES

Craniotomy will be performed on all subjects participating in this protocol. The surgical procedure is necessary component of clinical management. Patients will be under the care of the operating neurosurgeon of the Surgical Neurology Branch, NINDS.

3.6 COST AND COMPENSATION

3.6.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by insurance company. Medicines that are not part of the study treatment will not generally be provided or paid for by the NIH Clinical Center.

3.6.2 Compensation

Participants will not be compensated on this study.

3.6.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.7 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety evaluation approximately 30 days following the last dose of study therapy.

3.7.1 Criteria for removal from protocol therapy

- Completion of protocol therapy, craniotomy, PK and PD evaluations.
- Unacceptable Toxicity
- Investigator discretion
- Positive Pregnancy Test

3.7.2 Off-Study Criteria

- Screen failure
- Pathological evaluation demonstrates treatment effect (e.g. necrosis or inflammation as a result of therapy) rather than tumor recurrence, PK and PD evaluations will not be performed in these subjects. However, given presurgical exposure to LB100 patient will still be followed for AEs as noted in section **6.1**.
- Completed study follow-up period
- Participant requests to be withdrawn from study
- Loss of capacity to provide consent
- Investigator Discretion

4 CONCOMITANT MEDICATIONS/MEASURES

All participating subjects will undergo a craniotomy for resection of recurrent tumor. Concomitant medications/measures as clinically indicated will be delivered. The surgical team will provide routine care as needed. Participation in this study will not limit any routine care.

Only those medications that the patient is taking at baseline on a routine basis or medications that cause an AE will be captured in database. Thus one time medications and PRN medications will not be captured in database except those used to treat adverse events.]

5 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

5.1 BIOSPECIMEN COLLECTION

5.1.1 Pharmacokinetic/Pharmacodynamic Studies

Please note that tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.

5.1.1.1 Blood

Plasma PK: Plasma sampling for PK measurements of LB100 will be performed on all participating subjects. Blood samples for the determination of plasma levels of LB-100 and its metabolite LB100 M (endothall) will be obtained from each patient via 6mL chilled sodium heparin tube (BD, Franklin Lakes, NJ). At each time point, 6 mL will be drawn into a chilled heparin collection tube and kept on ice. Blood should be processed into plasma within 30 minutes of collection. Once plasma is aliquoted, 5N sodium hydroxide (NaOH) will be added at a ratio of 1:100 relative to plasma (e.g., 0.02 mL of 5N NaOH added to 2 mL plasma); the final NaOH concentration will be 50 mM in plasma. The fortified plasma will then be aliquoted into equal volumes in cryovials and frozen at -70° C until the time of analysis. Plasma PK evaluation will be performed in the Figg Laboratory as described in **5.1.3**

Blood samples will be collected at the following time points: 1*) Pre-dose; 2*) End of infusion (2 hours post-start); 3*) 30 minutes post LB100 infusion completion; 4*) 1 hour post LB-100 infusion completion; 5*) 2 hours post LB100 infusion completion; 6) 4 hours post LB100 infusion completion; 7) 8 hours post LB100 infusion completion; 8) Other blood samplings will take place at the time post-infusion tissues are retrieved. Note: * indicates that the sample may be obtained during the operative procedure. Later time points are after leaving the OR. All blood collection specified within time is allowed for +/- 15 minutes windows, with the exception of the pre-dose sample. The pre-dose blood sample may be collected any time within 48 hours prior to drug administration. Also, blood PK is at physician discretion. The primary purpose of the study is to determine the **tissue** PK (brain tumor).

Bioanalytical measurements will be conducted on an ultra HPLC-MSMS system using an assay developed and validated by the Clinical Pharmacology Program.

5.1.1.2 Tissue

Tissue PK: Glioma tissue sampling to detect and quantify LB100 will be performed post-dose in each patient as clinically indicated (time points from end of LB100 infusion will be recorded).

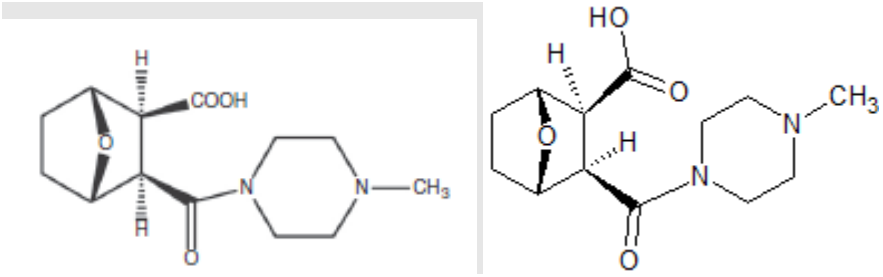
The removed tissues will be rinsed with saline immediately (to remove blood that contains LB100 to avoid falsely high measurements) then divided, flash frozen, and placed in a cryo tube. Majority of the tissue will be used for PK analysis. A small aliquot (~100 ug) will be saved in cryo tube for PD evaluation. PK samples will be transported to the Laboratory of Dr. Doug Figg (Molecular Pharmacology Section, Genitourinary Malignancies Branch) to quantitate LB100 tissue concentration.

5.1.2 Detailed PK Analysis

5.1.2.1 Overview:

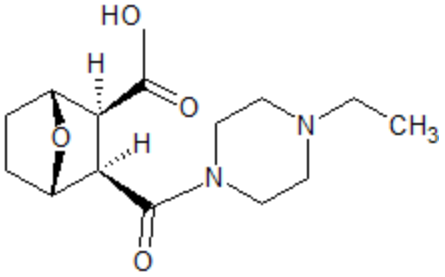
LC-MS-MS assay that can measure LB-100 in human plasma treated with 50 mM NaOH. Briefly, 50 µl of NaOH-treated plasma is extracted using a protein precipitation. Assay range is 2.0- 1000 ng/mL.

5.1.2.2 Analyte: LB-100 (Provided by the Pharmaceutical Collaborator)



Molecular Weight: 268.1 g/mol

5.1.2.3 Internal Standard: LB-105 (Provided by the Pharmaceutical Collaborator)



Molecular Weight: 282.2 g/mol

5.1.2.4 Conditions:

HPLC: Acquity UPLC (Waters Corp, Beverly, MA)
Binary Pump
Mobile Phase A1: 5 mM Ammonium Bicarbonate (aq)
Mobile Phase B1: Methanol
Mobile Phase A2: (90/10, v/v) Water/ACN
Mobile Phase B2: ACN
Strong Needle Wash: ACN
Weak Needle Wash: 5 mM Ammonium Bicarbonate (aq)
Seal Wash: (90/10, v/v) Water/ACN
Total run time: 3 mins

Gradient elution:

Time	**Flow Rate (mL/min)**	**%B2**
Initial	0.500	2.0

1.50	0.500	12.0
1.51	0.500	95.0
1.52	1.000	95.0
2.70	1.000	95.0
2.71	1.000	2.0
3.00	0.500	2.0

LB-100 and LB-105 retention time: 1.08 and 1.34 mins

5.1.2.5 Sample Manager

Injection volume: 5 µl
Sample temperature: 4 °C

5.1.2.6 Column Compartment

Column: Waters Acquity UPLC BEH C18 2.1x50mm, 1.7-um particle-size

Temperature: 50 °C

5.1.2.7 Mass Spectrometer: AB Sciex Q-Trap2 5500

Source Parameters

Mode: Electrospray Positive

Curtain gas: 30

5.1.2.8 CAD: Medium

5.1.2.9 IS: 5000

GS-1: 60

GS-2: 60

Source Temperature: 650 °C

MS Tune Parameters

Compound Name	**Parent (m/z)**	**Daughter (m/z)**	**DP**	**EP**	**CE**	**CXP**
LB-100	269.1	101.0	60	10	25	12
LB-105	283.2	115.1	60	10	25	12

5.1.2.10 Master Stock Solutions

5.1.2.10.1 Master Stock Preparation – LB-100 (1 mg/mL) from the Pharmaceutical Collaborator

a. Accurately weigh ~1-2 mg of LB-100 in a 5-mL glass centrifuge tube on the analytical balance.
b. Add 1 mL of 10mM NaOH (aq) for each mg of LB-100.
c. Vortex for 20 seconds.
d. Sonicate for 15 minutes at 35 °C, to ensure all drug is dissolved.
e. Transfer to an amber glass Waters HPLC vial and store at –80 °C.

5.1.2.10.2 Master Stock Preparation – IS (LB-105) – 1.00 mg/mL from the Pharmaceutical Collaborator

a. Accurately weigh ~1-2 mg of LB-105 in a 5-mL glass centrifuge tube on the analytical balance.
b. Add 1 mL of 10mM NaOH (aq) for each mg of LB-105.
c. Vortex for 20 seconds.
d. Sonicate for 15 minutes at 35 °C, to ensure all drug is dissolved.
e. Transfer to an amber glass Waters HPLC vial and store at –80 °C.

5.1.2.10.3 Solution Preparation

a. Mobile phase preparation
    i. A1: 5 mM Ammonium Bicarbonate (aq) – Dissolve 0.395 g of ammonium bicarbonate in 1L of Milli-Q water
    ii. A2 (Weak Wash/Seal Wash): 90:10 (v/v) Milli-Q H2O:ACN
    iii. B2 and Strong Wash: 100% ACN
b. 5 N NaOH
    i. Weigh out 20 grams of NaOH (pellets)
    ii. Dissolve in 80 mL of H_2O (initially…) with a stir bar on a stir plate
    iii. Once pellets dissolve and solution cools, then add 20 mL more (100 mL total volume)
c. 50 mM NaOH in human plasma preparation
    i. Transfer 25 mL of sodium heparin human plasma to a 50mL Falcon tube. Add 250 uL of 5 N NaOH using pipette. Mix thoroughly. Store at -20°C; the expiration date is that of the plasma
d. 10 mM NaOH (aq), master stock diluent, preparation
    i. Add 0.100 mL of 5N NaOH to 50 mL of DI water.
e. 10 mM TEA (aq) stock diluent preparation
    i. 0.5 M TEA (aq)
        1. Combine 1.4 mL of TEA and 18.6 mL of Milli-Q water in a falcon tube. Stir to mix and store the solution at room temperature. Expiration date is 1 month from preparation.
    ii. 10 mM TEA (aq)
        1. Combine 2 mL of 0.5 M TEA (aq) and 100 mL in an approximately sized storage bottle. Stir to mix and store the solution at room temperature. The expiration date is when the 0.5 M TEA expires.
f. 0.5% TEA in ACN, precipitation solution, preparation
    i. Add 0.250 mL of TEA to 50 mL of ACN.
g. 5 mM Ammonium Bicarbonate, reconstitution solution preparation
    i. Dissolve 0.395 g of ammonium bicarbonate in 1 L of Milli-Q water. Store at room temperature. The expiration date is 1 month from preparation.
h. Internal Standard – 200 ng/mL LB-105 in 10 mM TEA (aq)
    i. Add 100 uL of 1 mg/mL LB-105 to 900 uL 10 mM TEA to make a 100 ug/mL solution
    ii. Add 10 uL of 100 mg/mL LB-105 to 5 mL of 10 mM TEA (aq) to make a **200 ng/mL** IS-10mM TEA (aq) solution.
    iii. Discard solution after use.

5.1.2.10.4 Serial Dilutions and QC preparation

a. Pipette specified amount of stock into glass centrifuge tube. Add specified volume of diluent, 10 mM TEA (aq), by piston pipette. Vortex mix. Each stock is utilized to prepare the next dilution. Store at -20°C.

Stock Solution	Add X uL	of Stock:	Volume 10 mM TEA (aq) (uL)	Final volume (uL)	Working Stock Conc (ng/mL)	Final Conc (ng/ml)
X	100	**Master**	900	1,000	100,000	-
A	200	**X**	800	1,000	20,000	1,000
B	180	**X**	820	1,000	18,000	900
C	400	**B**	500	900	8,000	400
D	500	**C**	500	1000	4,000	200
E	200	**D**	800	1000	800	40
F	500	**E**	500	1000	400	20
G	250	**F**	750	1000	100	5
H	400	**G**	600	1000	40	2

5.1.2.10.5 Standard Curve

Concentration (ng/mL)	Add x µl	Of stock:	to 50 mM NaOH treated plasma µl:
1,000	10	A	190
900	10	B	190
400	10	C	190
200	10	D	190
40	10	E	190
20	10	F	190
5	10	G	190
2	10	H	190

***** Prepare curve in duplicate (50 uL each), plus 50 uL of blank human plasma for “Blank” and “IS-only” samples (in duplicate)

5.1.2.10.6 QC preparation

Prepare each QC batch by pipetting designated volume of stock solution into a 5 mL volumetric flask. Add blank human plasma until final volume is 5 mL. Mix well and aliquot 0.30 mL into clean, color-coded minifuge tubes (labeled with the date made) and store at –80 °C. (5 mL = 15 aliquots). Discard QCs older than 2 months.

QC Conc (ng/ml)	Stock	Volume Added	Actual Drug	Plasma up to-
6 (LQC; green)	F (400 ng/ml)	9 uL	3.6 ng	0.6 mL
40 (MQC; orange)	D (4,000 ng/ml)	5.0 uL	20 ng	0.5 mL
800 (HQC; red)	X (100,000 ng/ml)	4.0 uL	400 ng	0.5 mL
5,000 (DQC; blue)	X (100,000 ug/mL)	5.0 uL	500 ng	0.1 mL

* Prepare QCs in quintuplet (50 uL each). For the dilution (10-fold), add 5 uL of DQC to 45 uL of blank human plasma (in quintuplet).

5.1.2.10.7 Sample preparation

a. Pipette 50 µl of human plasma (standards, QCs, patient samples) into a labeled Eppendorf mini-centrifuge tube.
    - Curve in duplicate; QCs in quintuplet
b. For patient samples and DQC samples, first add 45 uL blank plasma, then 5 uL of sample
c. Add 50 ul of 100 ng/mL LB-105 in 10 mM TEA (aq) to each calibration, QC, blank with IS, and experimental sample. *Blanks get pure 10 mM TEA
d. Add 400 µl of 0.5% TEA in ACN to precipitate proteins.
e. Vortex for 30 sec to mix well.
f. Transfer ~450 uL into a clean, labeled 96-well Ostro plate.

g. Push supernatent through wells using Positive Pressure Manifold at ~12-15psi for 10 min.

h. Dry down under desiccated air in the TurboVap 96 (plate temp 40°C; gas flow ~30)
   - Once volume gets to ~100 uL remaining, add 300 uL more ACN to expedite evaporation

i. Reconstitute with 100 µL of 5 mM Ammonium Bicarbonate (aq) directly into collection plate.

j. Cover plate with parafilm and vortex for 20-30 sec.

k. Centrifuge plate for 10 min at 2,000 rpm at 4 C in big centrifuge.

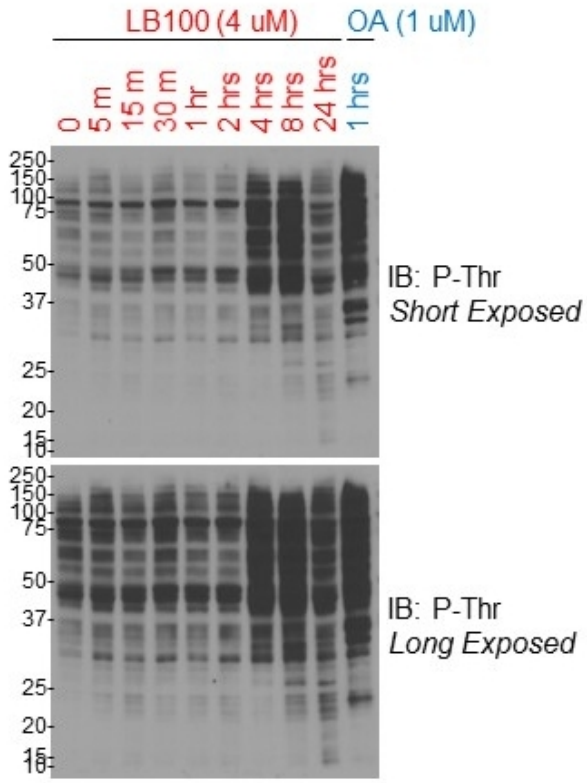
l. Run on UPLC-MS/MS

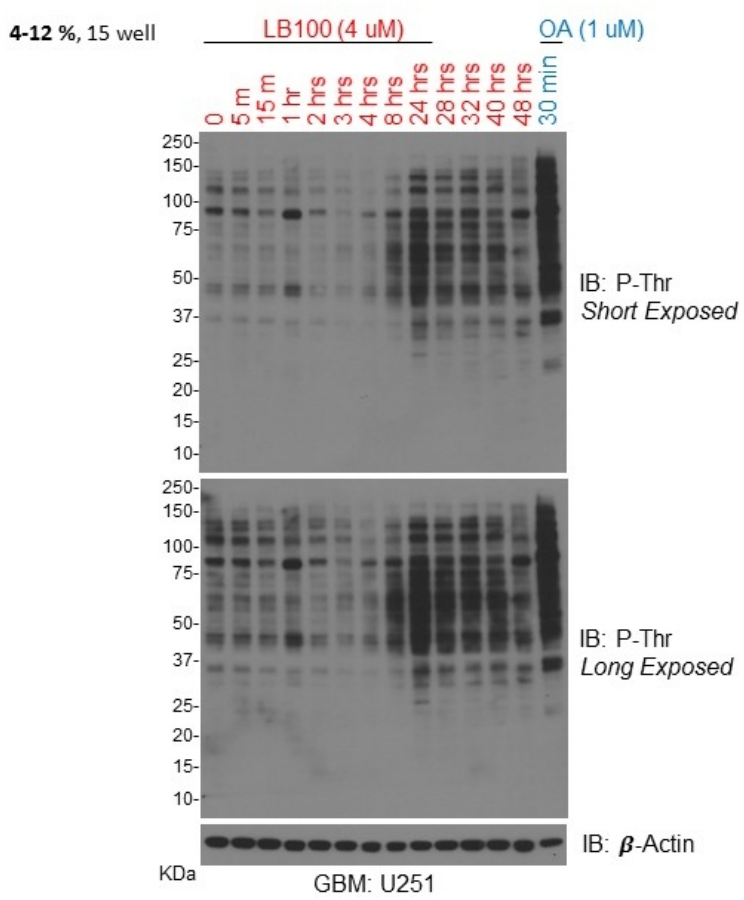
5.1.2.11 Assay Validation

See Appendix **16.3**

Tissue PD effect of LB100 will be performed by assaying phospho-protein expression in glioma tissue resected prior to and after infusion of LB100.

LB100 is a serine/threonine phosphatase inhibitor. Exposure to LB100 results in increased expression of phospho-serine/threonine residues. The antibody we chose to mark LB100 activity detects variety of proteins and peptides phosphorylated at the threonine residues in a manner independent of the surrounding amino acid sequence (Cell Signaling Technology, Phospho-Threonine Antibody (P-Thr-Polyclonal), #9381). Our preclinical studies indicate that expression of phospho-protein (phospho-threonine antibody was used) upon treatment with LB100 is seen at the earliest by 4 hours (Left panel below). By 8-hours expression is reliable, and maintained for approximately 40 hours (Right panel below). Okadaic acid (OA), well-established PP2A inhibitor, was used as positive control.


LB100 (4 uM)
OA (1 uM)
0
5 m
15 m
30 m
1 hr
2 hrs
4 hrs
8 hrs
24 hrs
1 hrs
250
150
100
75
50
37
25
20
15
10
IB: P-Thr
Short Exposed
IB: P-Thr
Long Exposed


4-12 %, 15 well
LB100 (4 uM)
OA (1 uM)
0
5 m
15 m
1 hr
2 hrs
3 hrs
4 hrs
8 hrs
24 hrs
28 hrs
32 hrs
40 hrs
48 hrs
30 min
250
150
100
75
50
37
25
20
15
10
IB: P-Thr
Short Exposed
IB: P-Thr
Long Exposed
IB: β-Actin
KDa
GBM: U251

5.1.2.11.1 Planned analysis

- PK evaluations of both blood and tumor tissues will be performed in Dr. Figg's Laboratory, and PD analyses of blood and tumor tissues in NOB Laboratory.
- A portion of blood collected for PK will be used to determine changes in phospho-protein expression in circulating PBMC.
- Intra-patient PD effect in PBMC (from above) and tumor tissue will be evaluated in all subjects for presence of correlation to identify potential predictive markers; this analysis consists of comparing PD effect in PBMC and tumor tissue to evaluate if PBMC changes predict effect in tumor tissue. Phospho-protein analyses (PD effect) of PBMC and tumor tissue will be performed in the NOB Laboratory.
- PD effect from LB100 treated tissues will only be evaluated with pathologic confirmation of recurrent tumor. Resected material demonstrating chemoradiation treatment effect or inflammatory response (pseudoprogression) will not be included in the analysis. This is because the compromised architecture of the BBB in necrotic tumor tissue may lead to falsely high penetration of LB100. These tissue PK/ PD and plasma PK specimens will be destroyed and the patient will be removed from the study.
- PK and PD specimens will be batched and performed retrospectively. The assays (both plasma and tissue) to quantitate LB100 and its major metabolite, LB100M (endothall) were developed in Dr. Figg's Laboratory. This data will be used to monitor LB-100 and its metabolite endothall plasma and tissue concentrations in order to correlate to pharmacodynamic endpoints, clinical response, and toxicity.
- No cell lines will be established.

5.1.3 Handling and Processing of Specimens

PK: The blood samples will be placed immediately on wet ice and refrigerated. The date and exact time of each blood draw should be recorded on the sample tube and the PK sheet. Tumor samples for PK and PD evaluations will be picked up from the Laboratory of Pathology by the NOB staff and transported to NOB Laboratory in Building 37. Tissues procured at various time points will be divided. Material for PD studies will be frozen in NOB Laboratory and remaining tissue for PK evaluation will be sent to the Figg Laboratory.

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

Upon arrival in the Clinical Pharmacology Program, samples will be centrifuged and the plasma transferred into cryovials for storage at -80 C until the time of analysis. In addition, samples will be barcoded as described in Section **5.2.2.1**.

5.1.3.1 PD: Tumor tissues will be frozen in a -80 freezer in the NOB Laboratory until time of analysis. For PD studies on blood, PBMC will be retrieved from the Figg Laboratory.

5.1.3.2 Tissue blocks can be shipped to Department of Pathology at NCI at the following address:

Laboratory of Pathology
Center for Cancer Research
National Cancer Institute
Building 10, Room 2S235
Bethesda, MD 20892-1500
Ph: 301-480-5010

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

5.2.1 Tissue blocks

5.2.1.1 Laboratory of Pathology

Paraffin embedded tissue block will be stored at Laboratory of Pathology located at building 10. Tissues designated for clinical diagnostics are transported to the Laboratory of Pathology (LP) where they are examined grossly and relevant portions are fixed, embedded in paraffin and sectioned and stained for diagnostic interpretation. Unutilized excess tissues are stored for up to three months, in accordance with College of American Pathologists/Joint Commission on Accreditation of Healthcare Organizations (CAP/JCAHO) guidelines, and then discarded. Following completion of the diagnostic workup, the slides and tissue blocks are stored indefinitely in the LP's clinical archives. All specimens are catalogued and retrieved utilizing the clinical laboratory information systems, in accordance with CAP/JCAHO regulations. The use of any stored specimens for research purposes is only allowed when the appropriate IRB approval has been obtained. In some cases, this approval has been obtained via the original protocol on which the patient was enrolled.

5.2.1.2 NOB Laboratory (PD Studies)

Samples will be ordered in CRIS and tracked through the Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH appropriate approvals and/or agreements, if required.

All samples sent to the Neuro-Oncology, Pathology Laboratory or the Brain Tumor Tissue Bank will be barcoded with data entered and stored in the Labmatrix system utilized by the CCR, NCI. This is a secure system with access limited to defined personnel.

Labmatrix creates a unique barcode ID for every sample which cannot be traced back to patients without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, date/time drawn, as well as box and freezer location. Patient demographics associated with the Clinical Center patient number are provided in the system. For

each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

These freezers are located onsite located in Room 1135, building 37. The freezer remains locked and access to stored clinical samples is restricted. Samples will be stored at -80^{o} C in freezers with electronic monitoring. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in the Labmatrix System. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with an IRB approved protocol and that any unused samples must be returned to the NCI. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

5.2.2 Pharmacokinetic samples

Samples for this study will be collected and stored by the Sample Processing Core. Bioanalytical measurements will be conducted on an ultra HPLC-MSMS system using an assay developed and validated by the Clinical Pharmacology Program.

Clinical Pharmacology Contact for Processing: NCIBloodcore@mail.nih.gov

5.2.2.1 Sample Data Collection

All samples sent to the Blood Processing Core (BPC) will be barcoded, with data entered and stored in Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined Figg lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number; time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following OHSRP/IRB approval of an additional protocol, granting the rights to use the material or if the use is not considered to be human subjects research.

5.2.3 Sample Storage and Destruction

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described above. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

If the patient withdraws consent the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document adverse events from the first study intervention, Study Day 1 through Study Day 30 (safety visit). Beyond 30 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

For patients whose pathological evaluation at craniotomy demonstrates treatment effect (e.g. necrosis or inflammation as a result of therapy) rather than tumor recurrence, adverse events will still be monitored from the first study intervention, Study Day 1 through Study Day 30 (safety visit).

Grade 1 events need not be captured, collected, or reported.

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **7.2.1**.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

- Coded, linked data in BTRIS (automatic for activities in the Clinical Center)
- Identified or coded, linked data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through:

- BTRIS (automatic for activities in the Clinical Center)
- Approved outside collaborators under appropriate individual agreements.
- Publication and/or public presentations.

When will the data be shared?

- Before publication.
- At the time of publication or shortly thereafter.

6.3 RESPONSE CRITERIA

This is not a therapeutic protocol. Response will not be monitored.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at:

https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING /IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to the Clinical Director/designee at NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NIH REQUIRED DATA AND SAFETY MONITORING PLAN

7.4.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis when patients are being actively treated on the trial to discuss each patient.

All data will be collected in a timely manner and reviewed by the principal investigator or associate investigators. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.4.2 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual NIH Intramural IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent

reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 SPONSOR PROTOCOL/SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2))

8.1.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse event (see **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.

- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 ASSESSMENT OF SAFETY EVENTS

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section **6.1.** All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor.

8.3 REPORTING OF SERIOUS ADVERSE EVENTS

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form.

All SAE reporting must include the elements described in **8.2**

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 SAFETY REPORTING CRITERIA TO THE PHARMACEUTICAL COLLABORATOR

Reporting will be per the collaborative agreement.

Reports should be sent to:
John Kovach, MD
Lixte
Email: jkovach@lixte.com

8.5 REPORTING PREGNANCY

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. Forms and instructions can be found here:
https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

8.5.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the pregnancy become known.

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (**8.1.2**) should be reported as SAEs.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

8.5.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 30 days after the last dose of LB100.

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or

congenital abnormality) occurring from the date of the first dose until 30 days after the last dose should, if possible, be followed up and documented.

8.6 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

8.7 SPONSOR PROTOCOL DEVIATION REPORTING

A Protocol Deviation is defined as any non-compliance with the clinical trial Protocol, Manual of Operational Procedures (MOP) and other Sponsor approved study related documents, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol deviation identified by the Staff or the site Monitor in the CCR Protocol Deviation Tracking System (PDTS) online application. The entries into the PDTS online application should be timely, complete, and maintained per CCR PDTS user requirements.

In addition, any deviation to the protocol should be documented in the participant's source records and reported to the reviewing IRB per their guidelines. OSRO required protocol deviation reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure:

- that the rights of the participants are protected;
- that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures; and
- the quality and integrity of study data and data collection methods are maintained.

Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Sponsor and Regulatory Oversight Support (SROS) Services contractor. Clinical site monitoring activities will be based on OSRO standards, FDA Guidance E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human

subject protections and integrity of the study data. OSRO will determine the intensity and frequency of monitoring based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. The Sponsor will conduct a periodic review of the CMP to confirm the plan's continued appropriateness. A change to the protocol, significant or pervasive non-compliance with GCP, or the protocol may trigger CMP updates.

OSRO SROS Monitoring visits and related activities will be conducted throughout the life cycle of each protocol. The first activity is before the study starts to conduct a Site Assessment Visit (SAV) (as warranted), followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will occur at the study site(s). Monitoring visit reports will describe visit activities, observations, and associated action items or follow-up required for resolution of any issues, discrepancies, or deviations. Monitoring reports will be distributed to the study PI, NCI CCR QA, CCR Protocol Support Office, coordinating center (if applicable), and the Sponsor regulatory file.

The site Monitor will inform the study team of any deviations observed during monitoring visits. If unresolved, the Monitor will request that the site Staff enter the deviations in the CCR Protocol Deviation Tracking System (PDTS) for deviation reporting to the Sponsor and as applicable per institutional and IRB guidance.

10 STATISTICAL CONSIDERATIONS

The primary objective of this study is to determine the PK response of LB100 in glioma tissue. The PK response is defined as a binary endpoint indicating the presence/absence of LB100, and will be established by measuring the level of LB100 in resected tumor tissues. This two-stage study is designed to define a significant PK effect with an expected target PK response rate of 40% across patients. The expected 40% response rate is based on preliminary in vitro and animal studies.

Up to 25 patients may be enrolled to obtain 8 evaluable patients. Five patients will be initially treated. If one of five demonstrates a PK response, three additional subjects will be enrolled. PK effect will be declared to be significant if at least 2 of the 8 patients demonstrate a PK response. This yields 87% power to detect a 40% PK response rate across patients, with an overall 10% false positive rate under the null hypothesis that the agent has no biologic effect(17).

The PK parameters, including C_{max}, AUC and elimination half-life time $t_{1/2}$, will be estimated based on standard PK modeling.

PD response is defined as statistically significant elevation of phospho-protein expression in treated tumor tissues compared to untreated glioma specimens. Tissue samples obtained with the initial surgical exposure of the tumor OR material obtained prior to LB100 infusion will be used as control. Untreated inter-patient baseline variance of phospho-protein expression and standard deviation (SD) will be calculated. Post-treatment PD effect (as measured by increase in tumor tissue phospho-protein expression) difference greater than 2.5 times the baseline SD is statistically significant at the .05 significance level. Due to relatively small sample size, t-

distribution is to be used to calculate the cutoff defining the PD response. The frequency of PD response and its 95% confidence interval will be estimated.

11 COLLABORATIVE AGREEMENTS

11.1 AGREEMENT TYPE – CRADA

The study agent, LB100 is provided by Lixte Biotechnology Holdings under a Cooperative Research and Development Agreement (CRADA), No. 03152. Results or study data will be communicated to CRADA partner, Lixte Biotechnology, according to the terms of the CRADA.

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

This study was designed to include women and minorities, but was not designed to measure differences of intervention effects. Males and females will be recruited with no preference to gender. No exclusion to this study will be based on race. Minorities will actively be recruited to participate.

12.2 PARTICIPATION OF CHILDREN

Because no dosing or adverse event data are currently available on the use of LB100 in subjects <18 years of age, children are excluded from this study.

12.3 PARTICIPATION OF SUBJECTS UNABLE TO CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. In such cases, subjects would be removed from the study as there is no prospect of direct benefit from research participation.

12.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

12.4.1 Risks

12.4.1.1 LB100

The primary risk to patients participating in this research study is from toxicity of the LB100 as described in the protocol. This is a Phase II clinical study to determine the PK/PD of LB100 in subjects with gliomas. Although safety of LB100 has been established from a Phase I study, LB100 has not been given to patients with gliomas. Potential risks include unexpected toxicity from LB100 infusion not seen during the phase I study. All patients will be carefully monitored during the post-operative period to identify potential toxicity. If patients experience unexpected toxicity, immediate medical treatment is available at the Clinical Center of NIH. LB100 may involve unpredictable risks to the participants.

12.4.1.2 Gd-DPTA enhanced MRI

There are no long-term risks of MRI scans, however, there is a risk of injury for persons who have metal in their bodies. Per Clinical Center practice, participants will be screened to determine whether they are at risk.

The MRIs to be done in this study will involve contrast. The risks associated with MRIs and contrast are discussed in the consent form.

The risks of an IV catheter include bleeding, infection, or inflammation of the skin and vein with pain and swelling.

Mild symptoms from gadolinium infusion occur in fewer than 1% of those who receive it and usually go away quickly. Mild symptoms may include coldness in the arm during the injection, a metallic taste, headache, and nausea. In an extremely small number, fewer than one in 300,000 people, more severe symptoms have been reported including shortness of breath, wheezing, hives, and lowering of blood pressure. Study participants should not receive gadolinium if they previously had an allergic reaction to it. Study participants will be asked about such allergic reactions before gadolinium is given. People with kidney disease are at risk for a serious reaction to gadolinium contrast called "nephrogenic systemic fibrosis (NSF)". This condition always involves the skin and can also involve the muscles, joints and internal organs. NSF has resulted in a very small number of deaths. A blood test of their kidney function may be done within the month before an MRI scan with gadolinium contrast. Study participants will not receive gadolinium for a research MRI scan if their kidney function is below the safe level. Most of the gadolinium contrast leaves the body in the urine. However, the FDA has issued a safety alert that indicates small amounts of gadolinium may remain in the body for months to years. The effects of the retained gadolinium are not clear. At this time, retained gadolinium has not been linked to health risks in people whose kidneys work well. Some types of gadolinium contrast drugs are less likely to remain in the body than others.

Additionally, the gadolinium contrast drugs that are used are less likely to remain in the body, whenever possible. The study participant will also be given additional information called a "Medication Guide." Upon request, the study participant will be given individual information about retained gadolinium we see on your studies.

Although MR imaging with gadolinium has been used as part of the standard care for patients with high grade gliomas, long term toxicities of gadolinium as a consequence of gadolinium accumulation in the body have been reported. All investigators will ascertain the MR imaging history of all prospective study participants to determine the extent of prior exposure to gadolinium contrast. Radiology will be consulted if there is a history of repeated gadolinium exposure.

12.4.1.3 CT scan

The risk of CT scan includes radiation risk which is listed in **12.4.1.4.** As noted in Section **2.2.2**, an MRI is preferred over CT Scan.

12.4.1.4 Radiation

The amount of radiation received from the CT Scan of the cerebrum in this study is 0.1 rem of radiation per year. The amount of radiation received in this study does not exceed the dose guideline established by the NIH Radiation Safety Committee for research subjects. The guideline is an effective dose of 5 rems (or 5,000 mrem) received per year. More information about radiation is available in the pamphlet, An Introduction to Radiation for NIH Research Subjects.

12.4.1.5 Blood collection

The risks of taking blood may include fainting, pain and/or bruising. Rarely, subjects may experience clotting or infection at the puncture site.

12.4.1.6 EKG

Other than the minor skin irritation from the electrodes there are no expected risks related to the EKG.

12.4.2 Benefits

There are no direct benefits to participating subjects.

12.4.3 Alternative Approaches or Treatments

Patients may have their planned craniotomy while not participating in this trial.

12.4.4 Procedure for Protecting Against or Minimizing any Potential Risks

All care will be taken to minimize side effects, but they can be unpredictable in nature and severity. This study may involve risks to patients, which are currently unforeseeable. All patients will have blood tests, examinations and scans as described in the study calendar (Section **3.4**). Patients will also be required to have a local physician to provide long-term care and to monitor for complications. If patients suffer any physical injury as a result of the participation in this study, immediate medical treatment is available at the Clinical Center, National Cancer Institute, Bethesda, Maryland. Although no compensation is available, any injury will be evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

12.5 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms) per discretion of the designated study investigator and with the agreement of the participant. Whether in person or

remote, the privacy of the subject will be maintained. Consenting investigators (and participant, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant.

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location but is not required.

Both the investigator and the participant will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found at:
https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

12.5.1 Request for Waiver of Consent for Screening Activities

Prior to the subject signing the consent for this study pre-screening activities listed in section **2.2.1** may be performed.

We request a waiver of consent for these activities as they involve only minimal risk to the subjects. A waiver will not adversely affect the rights and welfare of the subjects given that the activities are only intended to determine suitability for screening for participation in research protocols. These activities could not practicably be carried out without the wavier as central recruiting services, utilized in the NIH Clinical Center, perform pre-screening activities for multiple studies and obtaining consent for each one is beyond their resources. The subjects will be provided with additional pertinent information after participation as they will be informed whether or not they are eligible to sign a consent for additional screening.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or

termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, the Investigational New Drug (IND) and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

13.2 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted, and data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.3 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute has established policies and procedures for all study group members to disclose all

conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

13.4 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site(s) and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 PHARMACEUTICAL INFORMATION

14.1 LB100 (IND# 132947)

14.1.1 Source

Lixte Biotechnology Holdings, Inc.

14.1.2 Toxicity

Mice: In the mouse, 1.5 mg/kg (~4.3 mg/m2) was tolerated IP daily for up to 20 days without observable toxicity. Doses of 2.0 mg/kg IP daily for 5 days, were also explored, but although this dose was tolerated, the animals were lethargic and appeared ill. Therefore, for activity studies, the standard regimen used in numerous studies was no more than 1.5 mg/kg daily for 5 days. Although LB100 at 1.5 mg/kg IP daily for 5 days was well tolerated as a single agent, when given in combination with temozolomide in xenograft studies, mortality was observed in 1 of 10 mice.

Dose Ranging Study in Rats: A dose ranging study of single dose of LB100 was conducted in rats (12 male and 12 female) to examine systemic toxicity and to determine the maximum tolerated dose following a single intravenous (IV) dose of LB100. No toxicity was observed and no change in body weight related to drug treatment occurred.

Dose levels explored were 0.375, 0.75, 1.25, and 1.5 mg/kg. On Day 1, animals (n=3/sex/group) received LB100 prepared in phosphate buffered saline and deionized water (PBS/DI-Water) at the listed dose levels. Doses were delivered into a tail vein using a dose volume of 1 mL/kg. Body weights were collected on Days 1 (prior to dosing), 4, and 8 (prior to euthanasia). Mortality and moribundity checks were conducted twice daily, and detailed clinical observations were collected daily on Days 1 (prior to dosing and approximately two hours after dosing) through 8 (prior to euthanasia). Following collection of final body weight and detailed clinical observations, animals were euthanized without further observation. Over the course of the study, no clinical signs of toxicity, including changes in body weight, were observed in any treatment group. Thus, under the conditions of this study, LB100 treatment did not result in systemic toxicity, and a maximum tolerated dose was not determined.

Non-GLP Toxicity Study in Rats: A non-GLP dose ranging study of LB100 toxicity in the rat was conducted by the National Cancer Institute as part of the NExT Program. The study evaluated the toxicity of four consecutive daily IV infusions of LB100 in male Fischer rats. Twenty-four rats (6/group) were assigned to four treatment groups: vehicle control of phosphate buffered saline, 0.5, 0.75, and 1.5 mg/kg/day of LB100. Animals were weighed daily prior to dose administration. Clinical observations were recorded for all animals twice daily and again prior to termination.

Blood samples were collected in 3 of the 6 rats per dose group to evaluate hematology and clinical chemistry at 1 hour after the end of the infusion on day 2 and day 4 of treatment. On day 4, the rats were sacrificed approximately 1 to 2 hours after the last infusion by humane euthanasia and necropsied. Microscopic evaluation was performed on limited tissues.

There were no unscheduled deaths in any of the treatment groups. There were no effects on body weight and no clinical signs of toxicity in rats receiving 0.5 mg/kg/day. At 0.75 mg/kg/day, no clinical signs of toxicity were observed, however, a minimal decrease (1.6%) in body weight in comparison to day 1 was observed in 2 of 3 rats at termination of the study. At 1.5 mg/kg/day, clinical observations included blood in urine, lethargy and hind limb paresis. Lethargy was observed in all three rats on days 3 and 4, hind limb paresis was observed on day 4, and blood in the urine was observed in 2 of 3 rats on day 4. In addition, mean body weight was statistically significantly decreased between days 3 and 4 in comparison to the control group for the 1.5

mg/kg/day group. A mean decrease (23%) in body weight in the 1.5 mg/kg/day dose group was also observed on day 4 when compared to day 1 body weights.

Daily administration of LB-100 at 1.5 mg/kg/day for four consecutive days caused adverse effects in kidney (nephrosis) in the distal convoluted tubules in 3 of 3 rats; in the 0.75 mg/kg/day group, nephrosis was mild, and in the 0.5 mg/kg/day group, nephrosis was minimal.

Heart toxicity (epicardial hyperplasia with inflammation primarily on the epicardium of the atria) was observed in the 0.75 and 1.5 mg/kg/day groups. The hyperplasia was accompanied by subepicardial accumulation of mononuclear cells and eosinophils. One rat in the 1.5 mg/kg/day group had a large focus of inflammation with eosinophils associated with the aorta.

Rats in the 1.5 mg/kg/day group had compound-related lesions in femoral bone (necrosis), liver (mesothelial hyperplasia in 3 of 3 rats) and urinary bladder (mucosal erosion with transmural hemorrhage). Blood in the urine was also noted in 2 of 3 rats in the 1.5 mg/kg/day group. Primary clinical signs of blood in the urine and clinical chemistry findings of increased blood urea nitrogen and creatinine supported kidney and urinary bladder as target organs of toxicity. Hind leg paresis was observed in rats at the 1.5 mg/kg/day dose level; there were no histopathology correlates that would explain the paresis. Other changes observed in the 1.5 mg/kg/day group included significant weight loss, higher total white blood cell count, and eosinophil, basophil, lymphocyte, and monocyte counts, and a decrease in the percent of reticulocytes and platelet counts (day 4).

A NOAEL was not established for this 4-day intravenous toxicity study in male Fischer rats. The MTD for LB-100 in this study was 0.75 mg/kg/day. Kidney, heart, femoral bone, liver, and urinary bladder toxicity appeared to be dose-limiting toxicities in rats treated with LB-100 when administered intravenously once per day for four consecutive days.

GLP Repeat-Dose Toxicity Study in Rats: The objective of this study was to assess the potential toxicities of the test article, LB-100, when administered as a slow bolus intravenous injection to Sprague Dawley rats daily for 5 consecutive days, as well as to determine the reversibility of any potentially adverse findings during a minimum 23-day recovery period.

LB-100 in a 4.2% sterile sodium bicarbonate vehicle was administered by intravenous slow bolus push via infusion pump over an approximate 2-minute period once daily for 5 consecutive days to 3 toxicology groups (Groups 2-4) and 3 toxicokinetic groups (Groups 2A-4A) of Crl:CD(SD) rats. Dose levels were 0.5, 0.75, and 1.25 mg/kg/day for Groups 2/2A, 3/3A, and 4/4A, respectively. Concurrent control groups (Groups 1 and 1A) received the vehicle on a comparable regimen. The dose volume was 1.25 mL/kg for all groups. Groups 1-4 each consisted of 15 animals/sex. Group 1A consisted of 3 animals/sex, and Groups 2A-4A each consisted of 9 animals/sex. Following 5 days of dose administration, 10 rats/sex per toxicology group were euthanized; the remaining 5 rats/sex per toxicology group were euthanized following a minimum 23-day non-dosing (recovery) period.

All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed approximately weekly. Individual body weights were recorded daily during the dosing period and approximately weekly during the recovery period. Individual food consumption was recorded approximately weekly (with the exception of study weeks 3 and 4). Ophthalmic examinations

were performed on study days -2, 4, and 23 for males and -3, 4, and 22 for females. Clinical pathology parameters (hematology, coagulation, serum chemistry, and urinalysis) were analyzed for rats assigned to the primary (study day 5) and recovery (study days 29 and 28 for males and females, respectively) necropsies. Complete necropsies were conducted on all animals, and selected organs were weighed at the scheduled necropsies. Selected tissues were examined microscopically from all animals. For toxicokinetic evaluation, blood samples were collected from 3 animals/sex/group approximately 30 minutes following dose administration (Group 1A) and prior to dose administration, approximately 15 and 30 minutes following dose administration, and approximately 1, 4, and 12 hours following dose administration (Groups 2A-4A) on study days 0 and 4. All animals were euthanized and discarded on study day 6 (males) or 5 (females).

There were no test article-related effects on survival, clinical observations, or ophthalmic parameters. Test article-related lower cumulative body weight gains and food consumption values were noted during the dosing period for the 1.25 mg/kg/day group males and/or females when compared with the control group but were reversible following a minimum 23-day non-dosing (recovery) period. Changes in laboratory parameters were observed in the 1.25 mg/kg/day group. Hematological changes included test article-related higher lymphocyte counts in males and females on study day 5 versus control animals. Also observed in the 1.25 mg/kg/day group were minimally higher basophil counts, which were thought to be possibly test article-related, though not biologically meaningful, in males and females. Slightly higher counts of large unstained cell (LUC) counts were observed in the females on study day 5. Slightly lower reticulocyte counts, considered to be likely related to the test article, occurred in males and females of the 1.25 mg/kg/day group. In general, the hematologic changes resolved during the recovery period, with the exception of partially resolved higher lymphocyte and leukocyte counts in females.

The following abnormalities in clinical chemistry parameters were observed in the 1.25 mg/kg group: test article-related higher urea nitrogen, creatinine, phosphorus, and globulin values in males and/or females on study day 5. Test article-related minimally lower albumin levels and lower A/G ratio values occurred in females. These effects resolved during the recovery period. Slightly higher cholesterol values were also noted in the males on study day 5. The higher cholesterol values may have been test article-related. Higher gamma glutamyltransferase (GGT) values were noted in some males and females of the 1.25 mg/kg/day group, but it was not determined if the change was test article-related.

Test article-related effects on urinalysis parameters were observed in all treatment groups and included an increase in incidence and severity of: urine occult blood in 0.5 mg/kg/day group males and 0.75 and 1.25 mg/kg/day group males and females, urine protein in 1.25 mg/kg/day females, and increase in microscopic observations of leukocytes in males and females of the 1.25 mg/kg/day group, and in one female in both the 0.5 and 0.75 mg/kg/day group on study day 5. These changes were reversible.

The following macroscopic pathology findings were reported in the 1.25 mg/kg/day group: test article-related pale kidneys in males at the primary necropsy, as well as test article-related higher kidneys weights in males and females. At the recovery necropsy, kidney weights remained higher in 1.25 mg/kg/day group males.

Evaluation of microscopic pathology revealed adverse, test article-related nephrosis of the kidneys in the 0.75 and 1.25 mg/kg/day group males and females, which persisted or progressed in the males at the recovery necropsy. Minimal to severe nephrosis was observed in the kidney and was characterized by tubular degeneration and regeneration in the cortex and/or medulla. Minimal to mild nephrosis was most often observed in the medulla and/or occasionally the cortex. The 0.75 and 1.25 mg/kg/day group males had minimal to mild dilatation of the renal pelvis. The nephrosis correlated to pale kidneys in the males; higher kidney weights in the 1.25 mg/kg/day group males and females; higher serum urea nitrogen, phosphorous, and creatinine levels, and higher incidence of urine occult blood, protein, and leukocytes in the 1.25 mg/kg/day group males and/or females. Moderate nephrosis in the cortex and medulla persisted in the 0.75 and 1.25 mg/kg/day males and was accompanied by moderate interstitial fibrosis at the recovery necropsy and was associated with higher kidney weights in the 1.25 mg/kg/day group males at the recovery necropsy. Neither nephrosis nor interstitial fibrosis was observed in the females at the recovery necropsy.

LB-100 administration resulted in subacute subepicardial inflammation and/or mesothelial hypertrophy in the atria of males at ≥ 0.5 mg/kg/day and at 1.25 mg/kg/day in the females at the primary necropsy and was considered adverse in one 1.25 mg/kg/day group male. Minimal to mild subacute inflammation was observed in the epicardium and subepicardium of the left and/or right atrium of the heart in the 0.5, 0.75, and 1.25 mg/kg/day group males and the 1.25 mg/kg/day group females. One male in the 1.25 mg/kg/day group had mild subacute inflammation that was accompanied by minimal fibroplasia (plump fibroblasts) in the right atrium. Inflammation was often accompanied by mesothelial hypertrophy. There was a higher incidence of mesothelial hypertrophy in the 1.25 mg/kg/day group females when compared to the control group. Mesothelial hypertrophy was observed in control and test article-treated males and did not show a dose response.

Based on these findings, the severely toxic dose in 10% of the animals (STD 10) for this study was determined as 0.75 mg/kg/day. This dose corresponds to AUC_{last} values of 596 and 691 ng**.**h/mL and C_0 values of 1804 and 2347 ng/mL for males and females, respectively, on study day 4.

Dose Ranging Toxicity Studies in Dogs: The objectives of this study were to evaluate the tolerability and potential toxic effects of LB-100 when administered by intravenous (slow bolus) injection to beagle dogs in an escalating dose design. LB-100, in 4.2% sterile sodium bicarbonate, pH 8.4, was administered by intravenous injection (slow bolus push) to beagle dogs at dose levels of 0.1, 0.25, 0.5, and 1.0 mg/kg administered on study days 0, 4, 8, and 12, respectively, to Groups 1, 2, 3, and 4, respectively. The dose volume was 0.5 mL/kg for all groups. Each group consisted of 2 animals/sex. Each dose administration was followed by a 3-day washout period.

The animals were observed twice daily for mortality and morbidity. Clinical examinations were performed daily, and detailed physical examinations were performed approximately weekly during the pretest period, at the time of animal selection for randomization, and on the days of dosing, prior to each dose. Individual body weights and food consumption were recorded daily.

There were no LB-100-related effects on survival. A possible test article-related clinical observation of intermittent tremors was noted in one female on study day 13 following administration of LB-100 at 1.0 mg/kg. Lower body weight gains and food consumption were noted in one or both females during study days 8-16, following test article administration at 0.5 and 1.0 mg/kg. Based on the results of this study, LB-100 administered intravenously (slow bolus push) to beagle dogs at dose levels of 0.1, 0.25, 0.5, and 1.0 mg/kg every 4 days x 4 doses, resulted in a no-observed-effect level (NOEL) of 0.25 mg/kg. At dose levels of 0.5 and 1.0 mg/kg, lower body weight gains and food consumption were noted in females.

GLP Repeat-Dose Dog Toxicity Study: The objective of this study was to assess the potential toxicities of the test article, LB-100, when administered as a slow bolus intravenous injection to beagle dogs, daily for 5 consecutive days, as well as to determine the reversibility of any potentially adverse findings during a minimum 23-day recovery period.

LB-100, in 4.2% sterile sodium bicarbonate, pH 8.4, was administered via intravenous (slow bolus) push. Dose levels of 0.15, 0.30, and 0.75 mg/kg/day were administered on study days 1 to 5, to groups comprised of 10 dogs, 5 females and 5 males. The intent was to sacrifice 3 animals of each sex in each group the day following the fifth dose and 2 animals of each sex 23 days after the fifth dose. One male and one female were found dead the morning following the third injection at the highest dose, 0.75 mg/kg. As a result, the protocol was amended to decrease the highest dose level from 0.75 to 0.5 mg/kg for each of the remaining eight animals in the highest dose group. There were no further deaths. Note that a dose of 0.75 mg/kg in the dog (average weight of 9 kg and BSA of 0.5 m^2) is about 13.8 mg/m^2 or more than twice the MTD in the rat. This highest dose was selected because the dose range study in the dog revealed almost no signs of toxicity following a single dose of 1.0 mg/kg (approximately 18 mg/m^2) in the dose ranging study.

All animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily, and detailed physical examinations were performed approximately weekly. Individual body weights were recorded daily on the days of dosing and approximately weekly during the recovery period. Food consumption was recorded daily. Clinical pathology parameters (hematology, coagulation, serum chemistry, and urinalysis) were analyzed for all animals prior to randomization (study day -3) and for all surviving animals prior to the scheduled primary (study day 5) and recovery (study day 29) necropsies. Blood samples for toxicokinetic evaluation were collected from all animals prior to dosing, approximately 15 and 30 minutes following dose administration, and approximately 1, 4, and 12 hours following dose administration on study days 0 and 4. Ophthalmic examinations were performed on study days -3, 3, and 28. Electrocardiograms, heart rate, waveform intervals (PR, QRS, RR, QT, and QTcV), and blood pressure were recorded on study days -8, 4 (approximately 2 to 4 hours following dose administration), and 27. Complete necropsies were performed on all animals, and selected organs were weighed at the scheduled necropsies. Selected tissues were examined microscopically from all animals.

Test article-related clinical observations for the animals that died early in the 0.75 mg/kg/day group included intermittent tremors, swollen forelimb(s), and/or emesis containing white material. Necropsy of the two animals revealed both animals had similar test article-related macroscopic and microscopic findings affecting the gastrointestinal tract, kidneys, injection sites,

spleen, larynx, lungs and/or liver. Test article-related macroscopic findings consisted of dark red discoloration of the kidneys, small spleens, and red discoloration (reddened mucosa or dark red areas) of various segments of the gastrointestinal tract in males and females. Although the most noteworthy findings (mitotic figures and single cell necrosis of the renal tubular epithelial cells from the outer medulla and cortex) would be associated with altered renal function, these findings were not considered fatal lesions; therefore, the cause of death for each animal was considered undetermined but directly attributed to test article administration. All other animals survived to the scheduled primary (study day 5) and recovery (study day 29) necropsies.

There were no ophthalmic findings or changes in electrocardiography parameters and blood pressures associated with test article administration in any treatment groups.

Animals in the 0.30 mg/kg/day group had the following clinical observations during the dosing period: reduced body weights and food consumption, intermittent tremors, hypoactivity, vocalization, thin body condition, labored respiration, clear material around mouth, abnormal excreta (decreased defecation, mucoid feces, and/or yellow or red mucoid feces), emesis (containing food or white material), pale gums, and/or swollen forelimb(s). Most of these clinical findings were more prevalent towards the end of the dosing period. Macroscopic examination revealed lower thymus weights in the 0.30 mg/kg/day group females. Also reported in the 0.30 mg/kg/day group were lower chloride and sodium levels and the presence of blood in the urine.

Test article-related histological changes at the study day 5 primary necropsy consisted of congestion, basophilic tubules, and nephrosis of the kidney in the 0.15 (females only) and 0.30, and/or 0.75/0.50 mg/kg/day group and inflammation of the vagina and cervix in the 0.30 and group females. Persistent test article-related changes in the kidneys were seen in the 0.30 mg/kg/day group males and females at the study day 29 recovery necropsy.

The surviving animals of the 0.75/0.5 mg/kg/day group exhibited test article-related clinical signs that included: reduced body weights and food consumption; also observed were impaired muscle coordination, intermittent tremors, hypoactivity, thin body condition, body and/or ear(s) cool to touch, increased respiration rate, green and red nasal discharge, clear and/or red material around nose or mouth and/or on ear(s), abnormal excreta (decreased defecation, mucoid feces, yellow and red mucoid feces, and diarrhea containing red material), emesis (containing food, white material, and/or yellow material), pale gums, and/or swollen forelimb(s).

During the recovery period, all surviving animals had body weight gains indicative of recovery, and the majority of the other observed clinical signs resolved within the first few days of the recovery period.

At the primary necropsy (study day 5), test article-related macroscopic findings consisted of dark red discoloration of the kidneys, small spleens, and red discoloration (reddened mucosa or dark red areas) of various segments of the gastrointestinal tract in the 0.75/0.50 mg/kg/day group males and females. At the recovery necropsy (study day 29), no test article-related macroscopic findings were observed.

Test article-related changes in organ weights at the study day 5 primary necropsy included the following: higher adrenal gland and kidney weights and lower spleen and thymus weights in the

0.75/0.50 mg/kg/day group males and females. At the recovery necropsy (study day 29), higher kidney weights were noted in the surviving male from the 0.75/0.50 mg/kg/day group.

Test article-related effects on hematology and coagulation parameters at the study day 5 evaluation included higher red blood cell mass (red blood cell count, hemoglobin, and hematocrit), lower platelet counts, and prolonged activated partial thromboplastin time values in the animals of the 0.75/0.50 mg/kg/day group. At the study day 29 evaluation, there were no residual effects of test article administration on hematology or coagulation parameters.

At the study day 5 evaluation, test article-related changes in serum chemistry parameters included higher urea nitrogen, creatinine, cholesterol, phosphorus, triglycerides, total bilirubin, alanine aminotransferase, aspartate aminotransferase, sorbitol dehydrogenase, and lower chloride and sodium levels in the 0.75/0.50 mg/kg/day group. At study day 29, higher cholesterol and triglycerides values were noted for the surviving male in the 0.75/0.50 mg/kg/day group compared to the control group; however, a decrease from the study day 5 values was present suggestive of recovery.

Test article-related changes in urinalysis parameters observed at the study day 5 evaluation included lower specific gravity, higher urine volume, and increased presence of blood in the 0.75/0.50 mg/kg/day groups. At study day 29, no test article-related changes in urinalysis parameters were present.

Test article-related histological changes at the study day 5 primary necropsy in the 0.75/0.50 mg/kg/day group consisted of: congestion, basophilic tubules, and nephrosis of the kidney; increased mitoses of the liver in males; lymphoid depletion or single cell necrosis of the lymph nodes, Peyer's patches, and thymus; hypocellular bone marrow; erosion and hemorrhage within the gastrointestinal tract; and inflammation of the vagina and cervix in the females. Persistent test article-related changes in the kidneys were seen in males and females at the study day 29 recovery necropsy.

In conclusion, administration of LB-100 via daily intravenous (slow bolus) injection for 5 days to male and female beagle dogs was well tolerated at the dosage level of 0.15 mg/kg/day. At dosage levels of 0.30 and 0.75/0.50 mg/kg/day, administration of LB-100 resulted in adverse clinical observations, lower body weights, and histological findings (congestion and nephrosis in kidneys, increased mitoses and single cell necrosis in liver, lymphoid depletion and single cell necrosis in thymus, and/or erosion and/or hemorrhage in stomach or intestines) correlating with effects on clinical pathology, organ weight, and/or macroscopic findings during the dosing period. Persistent adverse test article-related histological changes in the kidneys were observed in the 0.30 and 0.75/0.50 mg/kg/day group males and females at the day 29 recovery necropsy. These changes were more indicative of a progression towards chronicity rather than recovery. In addition, lethality was observed at 0.75 mg/kg/day. Therefore, the Highest Non-Severely Toxic Dose (HNSTD) was 0.15 mg/kg, which corresponded to an AUC_{last} for LB-100 of 267 and 335 ng•h/mL on study day 4 for males and females, respectively.

Summary of Results of Toxicity Studies: Results indicate that in the mouse, 1.5 mg/kg (approximately 4.7 mg/m^2) can be administered daily for 5 days (up to 20 days) without observable toxicity.

The results of the rat toxicity studies varied somewhat according to strain. In Fischer rats, the MTD was 0.75 mg/kg (about 4.5 mg/m^2) iv daily for 4 days. Administration of 1.5 mg/kg/day for 4 days caused renal and bladder toxicity and transient hind limb paresis with no gross or microscopic evidence of neurological tissue damage.

Administration of LB-100 via daily intravenous (slow bolus) injection for 5 days to male and female Sprague Dawley rats at dose levels of 0.5, 0.75, and 1.25 mg/kg/day resulted in adverse, test article-related nephrosis of the kidneys in the 0.75 and 1.25 mg/kg/day group males and females, which persisted or progressed in the 0.75 and 1.25 mg/kg/day group males at the recovery necropsy. LB-100 administration resulted in subacute subepicardial inflammation and/or mesothelial hypertrophy in the atria of males at $\geq$ 0.5 mg/kg/day and at 1.25 mg/kg/day in the females at the primary necropsy and was considered adverse in one 1.25 mg/kg/day group male. Based on these findings, the severely toxic dose in 10% of the animals (STD 10) for this study was determined as 0.75 mg/kg/day. This dose corresponds to AUC_{last} values of 596 and 691 ng.h/mL and C_0 values of 1804 and 2347 ng/mL for males and females, respectively, on study day 4.

Test article-related laboratory abnormalities were observed in rats administered LB-100 at the 1.25 mg/kg dose level in the GLP toxicity study. Hematologic changes included higher lymphocytes and leukocytes on day 5 that partially resolved in the recovery period. Blood chemistry changes included increased urea nitrogen, creatinine, phosphorus, globulin, and cholesterol on day 5; most of these parameters resolved in the recovery period. Urinalysis showed blood, protein, and leukocytes in urine on day 5, but these changes were reversible during recovery.

Administration of LB-100 via daily intravenous (slow bolus) injection for 5 days to male and female beagle dogs was well tolerated at the dosage level of 0.15 mg/kg/day. At dosage levels of 0.30 and 0.75/0.50 mg/kg/day, administration of LB-100 resulted in adverse clinical observations, lower body weights, and histological findings (congestion and nephrosis in kidneys, increased mitoses and single cell necrosis in liver, lymphoid depletion and single cell necrosis in thymus, and/or erosion and/or hemorrhage in stomach or intestines) correlating with effects on clinical pathology, organ weight, and/or macroscopic findings during the dosing period. Persistent adverse test article-related histological changes in the kidneys were observed in the 0.30 and 0.75/0.50 mg/kg/day group males and females at the day 29 recovery necropsy. These changes were more indicative of a progression towards chronicity rather than recovery. In addition, lethality was observed at 0.75 mg/kg/day. Therefore, the Highest Non-Severely Toxic Dose (HNSTD) was 0.15 mg/kg, which corresponded to an AUC_{last} for LB-100 of 267 and 335 ng•h/mL on study day 4 for males and females, respectively.

Test article-related laboratory abnormalities were observed in dogs administered LB-100 at the 0.75/0.5 mg/kg dose level in the GLP toxicity study. Hematologic changes included higher red blood cell mass (red blood cell count, hemoglobin, and hematocrit) and prolonged activated partial thromboplastin time (aPTT). Blood chemistry changes included increased urea nitrogen, creatinine, cholesterol, phosphorus, triglycerides and some hepatic enzymes on day 5; the changes showed evidence of resolving during recovery. Urinalysis on day 5 showed increased

presence of blood in urine, higher urine volume and lower specific gravity; these changes were not present at recovery.

14.1.3 Formulation and preparation

LB-100 for Injection is supplied as a sterile solution for intravenous administration. Each vial contains 10 mL of a 1.0 mg/mL solution of LB100 in monosodium glutamate, pH 10.5.

The appropriate dose of LB100 is to be drawn from the vial using a sterile syringe and added to normal saline (0.9%) before infusing intravenously. Dilution in saline was done to reduce the pH so that the infusate is non-irritating (but extravasation is to be avoided). However, intravenous infusion of LB100 diluted in 50-500 mL of normal saline and infused over 15 minutes to 120 minutes, respectively, produced no local irritation (unpublished data of Lixte)

14.1.4 Stability and Storage

LB100 is stored frozen at MRIGlobal, Kansas City, MO. Stability analysis is done annually and will be kept in the study/pharmacy records with the drug supply. Drug is shipped frozen and will be stored in the Clinical Center Pharmacy.

14.1.5 Administration procedures

The recommended dose of LB-100 for Phase 2 trials is based on the documented MTD dose of 2.33 mg/m^2 diluted in 500 mL normal sterile saline and infused over 2 hours daily for 3 consecutive days repeated every three weeks. In this Phase II study, we will administer a single dose of 2.33 mg/m^2 in 500 mL normal sterile saline infused over 2 hours (+/- 15 minutes).

14.1.6 Incompatibilities

None identified.

15 REFERENCES

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16 APPENDICES

16.1 PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

16.2 PK WORKSHEET

Cover Letter Form

PKs to Dr. Figg's Lab

Page: 102-11964

Samples must be retrieved and processed within 30 minutes.

PLEASE clearly label the PIVs for PK's/LB-100 infusion, respectively.

Protocol # 17-C-0037, Drug: LB-100

Cycle 1 Day 1 LB-100 Dose:_____

Staff Obtaining Samples: _____(printed name)

Signature/initials: _____

Staff Obtaining Samples: _____(printed name)

Signature/initials: _____

Staff Obtaining Samples: _____(printed name)
Signature/initials: _____

Staff Obtaining Samples: _____(printed name)
Signature/initials: _____

Staff Obtaining Samples: _____(printed name)
Signature/initials: _____

When documenting in CRIS, please include the details in your note:

- Pre-chilled 6 ml Sodium Heparin tube
- Date and time sample obtained
- If the collection time differs from the "scanned time," please indicate this in your note with actual time sample obtained.
- If sample or EKG is obtained outside of the protocol-defined time window (+/- 15 min), please indicate reason

- Update Research Nurse via phone or email if sample outside of the defined window.

DATE: _____ **Protocol # DRUG - LB-100**

PKs to Dr. Figg's Lab
Page: 102-11964

Clock Used: _____

Part 1
Cycle 1 Day 1 LB-100 Dose: _____ mg Ht: _____
Wt: _____
BSA: _____

Research Nurse: name
phone
beeper
PI: Eric Burton

You may draw PK's through a CVC; or, through a peripheral line (please note location of blood draw under "Comments")
** You must populate each "scheduled time cell" (after the Pre-infusion cell) using the EOI as reference as soon as EOI is determined. Two RNs must independently check your scheduled time column.

DO NOT DRAW PK'S THROUGH THE SAME LINE AS DRUG ADMINISTRATION.

Study Day	Time Interval	Amount/Type/Tube// Handling	Test	Schd Time (Military)	Actual Time (Military)	Special Instructions	Comments	Signature	Reason if missed	Documented in CRIS Serial Testing Note (initial)
	LB-100 Infusion Start Time:_____			LB-100 Infusion End Time:_____						
Day 1	pre LB-100	One 6 mL Sodium Heparin (Green Top) Date and time tube	PK			Page 102-11964 - Figg Lab Place on wet ice and refrigerate				
	End of Infusion (2hr post infusion start	One 6 mL Sodium Heparin (Green Top) Date and time tube	PK			Page 102-11964 - Figg Lab Place on wet ice and refrigerate				
	End of Infusion (2hr post infusion start	N/A	EKG			Please provide the EKG to the Research Nurse				
	30 min post EOI	One 6 mL Sodium Heparin (Green Top) Date and time tube	PK			Page 102-11964 - Figg Lab Place on wet ice and refrigerate				
	1hr post EOI	One 6 mL Sodium Heparin (Green Top) Date and time tube	PK			Page 102-11964 - Figg Lab Place on wet ice and refrigerate				
	2hr post EOI	One 6 mL Sodium Heparin (Green Top) Date and time tube	PK			Page 102-11964 - Figg Lab Place on wet ice and refrigerate				
	4hr post EOI	One 6 mL Sodium Heparin (Green Top) Date and time tube	PK			Page 102-11964 - Figg Lab Place on wet ice and refrigerate				
	At the time of tissue procurement in OR	Cryo tube	PD	N/A		Page 102-11964 - Figg Lab Place on wet ice and refrigerate				
	8hr post EOI	One 6 mL Sodium Heparin (Green Top) Date and time tube	PK			Page 102-11964 - Figg Lab Place on wet ice and refrigerate				

Please sign each time you draw a PK.
Please chart in CRIS the actual times the PK's were drawn

(patient label)

16.3 PK ASSAY VALIDATION

16.3.1 LB-100 Plasma

Sample Name	**Calculated Concentration**			
LB-100	Day 1	Day 2	Day 3	Day 4
H	**1.041**	1.989	1.885	1.87
G	4.458	4.873	5.084	5.448
F	20.07	20.78	20.66	20.69
E	40.68	40.99	41.20	42.32
D	202.50	210.30	192.60	213.00
C	402.90	401.70	379.60	404.10
B	814.60	875.50	885.90	828.10
A	957.40	968.20	938.90	962.70
LOQ	0.77	1.97	2.13	1.85
LQC	5.10	7.19	5.78	6.32
MQC	41.23	39.10	39.51	41.96
HQC	736.30	767.00	771.30	727.50
DQC	5121.00	5205.00	4954.00	4771.00
LOQ	2.06	1.94	1.76	1.90
LQC	5.06	8.02	6.20	6.41
MQC	39.66	40.16	39.30	40.93
HQC	754.20	780.20	770.00	730.60
DQC	4886.00	5244.00	4809.00	4810.00
LOQ	1.09	2.03	2.01	2.22
LQC	5.10	7.37	5.83	6.51

MQC	40.00	40.39	39.98	38.36
HQC	718.90	791.20	763.20	709.60
DQC	4890.00	5239.00	4876.00	5054.00
LOQ	2.08	2.20	1.96	2.12
LQC	5.38	7.56	5.90	6.11
MQC	40.32	41.91	39.57	40.98
HQC	743.10	800.90	766.90	735.40
DQC	5104.00	5378.00	4840.00	4721.00
LOQ	1.36	2.11	1.97	2.05
LQC	5.22	7.60	6.07	5.86
MQC	41.02	41.37	39.13	40.58
HQC	762.80	775.40	793.70	775.40
DQC	5005.00	5277.00	4893.00	4802.00
H	1.93	2.02	2.07	2.00
G	5.85	5.08	5.05	5.27
F	21.47	18.98	21.08	19.71
E	41.27	37.18	42.51	41.61
D	208.20	212.80	202.40	199.80
C	414.90	414.20	404.30	399.70
B	848.70	866.70	885.90	821.80
A	943.50	1001.00	974.30	933.20
	2/18/2016	**2/19/2016**	**2/22/2016**	**2/23/2016**
Linearity				
		AVG	SD	

Day 1	0.994049	0.99659875	0.001934316	
Day 2	0.998141			
Day 3	0.998061			
Day 4	0.996144			

Nominal (ng/mL)	**Day 1-1**	**Day 1-2**	**Day 2-1**	**Day 2-2**	**Day 3-1**	**Day 3-2**	**Day 4-1**	**Day 4-2**	**GM (ng/mL)**	**SD (ng/mL)**	**DEV (%)**	**R.S.D (%)**	**n**
2		1.93	1.99	2.02	1.89	2.07	1.87	2.00	1.97	0.07	-1.68%	3.75%	7
5.00	4.46	5.85	4.87	5.08	5.08	5.05	5.45	5.27	5.14	0.41	2.80%	7.96%	8
20.00	20.07	21.47	20.78	18.98	20.66	21.08	20.69	19.71	20.43	0.80	2.15%	3.92%	8
40.00	40.68	41.27	40.99	37.18	41.20	42.51	42.32	41.61	40.97	1.66	2.43%	4.04%	8
200.00	202.50	208.20	210.30	212.80	192.60	202.40	213.00	199.80	205.20	7.14	2.60%	3.48%	8
400.00	402.90	414.90	401.70	414.20	379.60	404.30	404.10	399.70	402.68	10.88	0.67%	2.70%	8
900.00	814.60	848.70	875.50	866.70	885.90	885.90	828.10	821.80	853.40	29.14	-5.18%	3.41%	8
1000.00	957.40	943.50	968.20	1001.00	938.90	974.30	962.70	933.20	959.90	22.05	-4.01%	2.30%	8

LLOQ QC	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**GM**	**SD**	**DEV**
2 ng/mL	0.77	1.97	2.13	1.85	1.88	0.377066079	-6.11%
	2.06	1.94	1.76	1.90			
	1.09	2.03	2.01	2.22			
	2.08	2.20	1.96	2.12			
	1.36	2.11	1.97	2.05			

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	
Between Groups	1.123	3	0.3744	F (3, 16) = 3.799	P = 0.0313	
Within Groups	1.577	16	0.09855			
Total	2.7	19				
	BRP	12.51				
	WRP	16.72				

Low QC	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**GM**	**SD**	**DEV**
6 ng/mL	5.10	7.19	5.78	6.32	6.23	0.90406471	3.84%
	5.06	8.02	6.20	6.41			
	5.10	7.37	5.83	6.51			
	5.38	7.56	5.90	6.11			
	5.22	7.60	6.07	5.86			

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	
Between Groups	14.66	3	4.886	F (3, 16) = 92.41	P < 0.0001	
Within Groups	0.846	16	0.05287			
Total	15.5	19				
	BRP	15.78				
	WRP	3.69				

Middle QC	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**GM**	**SD**	**DEV**
40 ng/mL	41.23	39.10	39.51	41.96	40.27	0.977607714	0.68%
	39.66	40.16	39.30	40.93			
	40.00	40.39	39.98	38.36			
	40.32	41.91	39.57	40.98			
	41.02	41.37	39.13	40.58			

ANOVA					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	4.06	3	1.353	F (3, 16) = 1.536	P = 0.2437
Within Groups	14.1	16	0.8811		
Total	18.16	19			
	BRP	0.76			

	WRP	2.33			

High QC	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**GM**	**SD**	**DEV**
800 ng/mL	736.30	767.00	771.30	727.50	758.68	25.75993625	-5.17%
	754.20	780.20	770.00	730.60			
	718.90	791.20	763.20	709.60			
	743.10	800.90	766.90	735.40			
	762.80	775.40	793.70	775.40			

ANOVA					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	7831	3	2610	F (3, 16) = 8.744	P = 0.0012
Within Groups	4777	16	298.5		
Total	12608	19			
	BRP	2.83			
	WRP	2.28			

Dilution QC	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**GM**	**SD**	**DEV**
5000 ng/mL	5121	5205	4954	4771	4993.95	195.6991448	-0.12%
	4886	5244	4809	4810			
	4890	5239	4876	5054			
	5104	5378	4840	4721			

	5005	5277	4893	4802			

ANOVA					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	7831	3	2610	F (3, 16) = 8.744	P = 0.0012
Within Groups	4777	16	298.5		
Total	12608	19			
	BRP	2.83			
	WRP	2.28			

Dilution QC	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**GM**	**SD**	**DEV**
5000 ng/mL	5121	5205	4954	4771	4993.95	195.6991448	-0.12%
	4886	5244	4809	4810			
	4890	5239	4876	5054			
	5104	5378	4840	4721			
	5005	5277	4893	4802			

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	
Between Groups	580675	3	193558	F (3, 16) = 21.07	P < 0.0001	
Within Groups	146990	16	9187			
Total	727665	19				

	BRP	3.85				
	WRP	1.92				

16.3.2 LB-100 Tissue

Sample Name	**Calculated Concentration**		
LB-100 tissue	Day 1	Day 2	Day 3
H	3.05	2.67	3.22
G	9.25	10.19	10.55
F	28.17	32.70	31.86
E	101.13	106.41	101.28
D	292.02	294.28	292.85
C	996.24	942.92	960.10
B	2905.33	2903.10	2879.97
A	10135.85	10350.06	9981.22
LOQ	2.89	3.21	2.66
LQC	7.03	8.17	7.00
MQC	1871.82	1876.91	1722.76
HQC	7756.64	7715.07	7816.03
LOQ	2.98	2.97	3.02
LQC	6.92	7.27	6.56
MQC	1849.08	1876.22	1816.34
HQC	8213.18	7792.95	7608.25
LOQ	1.80	3.57	2.64

LQC	6.40	7.88	6.91
MQC	1906.27	1790.11	1838.19
HQC	8234.50	8057.81	7662.84
LOQ	1.04	2.66	3.63
LQC	6.92	8.00	7.32
MQC	1935.47	1846.60	1827.07
HQC	8129.39	7774.61	7650.04
LOQ	4.21	4.97	2.73
LQC	6.84	7.45	7.61
MQC	1967.56	1787.57	1882.56
HQC	8266.66	7652.88	7927.77
H	3.02	3.26	2.68
G	**1.50**	9.96	10.27
F	30.41	33.43	31.29
E	112.10	97.67	100.82
D	301.06	285.99	292.89
C	1014.10	931.49	953.40
B	**1422.93**	2804.38	2873.89
A	**4232.95**	10256.63	10519.78
	4/27/2016	**5/2/2016**	**5/3/2016**
Linearity			
		AVG	SD
Day 1	0.997142045	0.996223902	0.001404329
Day 2	0.99460729		

Day 3	0.996922372		

Nominal (pg/mg)	**Day 1-1**	**Day 1-2**	**Day 2-1**	**Day 2-2**	**Day 3-1**	**Day 3-2**	**GM (ng/mL)**	**SD (ng/mL)**	**DEV (%)**	**R.S.D (%)**	**n**
3	3.05	3.02	2.67	3.26	3.22	2.68	2.98	0.26	-0.56%	8.59%	6
10.00	9.25		10.19	9.96	10.55	10.27	10.04	0.49	0.44%	4.89%	5
30.00	28.17	30.41	32.70	33.43	31.86	31.29	31.31	1.87	4.37%	5.96%	6
100.00	101.13	112.10	106.41	97.67	101.28	100.82	103.24	5.17	3.24%	5.01%	6
300.00	292.02	301.06	294.28	285.99	292.85	292.89	293.18	4.83	-2.27%	1.65%	6
1000.00	996.24	1014.10	942.92	931.49	960.10	953.40	966.38	32.08	-3.36%	3.32%	6
3000.00	2905.33		2903.10	2804.38	2879.97	2873.89	2873.33	40.95	-4.22%	1.43%	5
10000.00	10135.85		10350.06	10256.63	9981.22	10519.78	10248.71	205.00	2.49%	2.00%	5

LLOQ QC	**Day 1**	**Day 2**	**Day 3**	**GM**	**SD**	**DEV**
3 pg/mg	2.89	3.21	2.66	3.00	0.91963	-0.04%
	2.98	2.97	3.02			
	1.80	3.57	2.64			
	1.04	2.66	3.63			
	4.21	4.97	2.73			

ANOVA					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	2.019	2	1.009	F (2, 12) = 1.233	P = 0.3258
Within Groups	9.821	12	0.8184		
Total	11.84	14			
	BRP	6.51			
	WRP	30.17			

Low QC	**Day 1**	**Day 2**	**Day 3**	**GM**	**SD**	**DEV**
8 pg/mg	7.03	8.17	7.00	7.22	0.51941	-9.77%
	6.92	7.27	6.56			
	6.40	7.88	6.91			
	6.92	8.00	7.32			
	6.84	7.45	7.61			

ANOVA					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	2.316	2	1.158	F (2, 12) = 9.509	P = 0.0034
Within Groups	1.461	12	0.1218		
Total	3.777	14			
	BRP	6.31			

	WRP	4.83			

Middle QC	**Day 1**	**Day 2**	**Day 3**	**GM**	**SD**	**DEV**
2000 pg/mg	1871.82	1876.91	1722.76	1852.97	61.2912	-7.35%
	1849.08	1876.22	1816.34			
	1906.27	1790.11	1838.19			
	1935.47	1846.60	1827.07			
	1967.56	1787.57	1882.56			

High QC	**Day 1**	**Day 2**	**Day 3**	**GM**	**SD**	**DEV**
8000 pg/mg	7756.64	7715.07	7816.03	7883.91	234.934	-1.45%
	8213.18	7792.95	7608.25			
	8234.50	8057.81	7662.84			
	8129.39	7774.61	7650.04			
	8266.66	7652.88	7927.77			

ANOVA					
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Between Groups	429092	2	214546	F (2, 12) = 7.492	P = 0.0077
Within Groups	343626	12	28636		
Total	772718	14			
	BRP	2.45			

	WRP	2.15			