CLINICAL TRIAL PROTOCOL

Study Title:	A Randomized, Double-Blind, Placebo-Controlled, Study to Evaluate the Safety and Efficacy of LB1148 in Accelerating the Time to Return of Bowel Function in Subjects Undergoing Planned Bowel Resection (INTEGRITY)
Study Number:	PBI-POI-301
Study Phase:	3
Test Product:	LB1148
IND Number:	158008
Indication:	Accelerate the time to return of bowel function in patients undergoing abdominal surgery
Sponsor:	Palisade Bio, Inc. 5800 Armada Drive, Suite 210 Carlsbad, CA 92008
Study Director:	[redacted]
Medical Monitor:	[redacted]

Version	Date
Original Protocol, v1.0	February 10, 2022
Protocol v2.0	March 9, 2022
Protocol v3.0	May 20, 2022
Protocol v4.0	June 10, 2022

Confidentiality Statement

The information in this document is confidential and will not be disclosed to others without written authorization from **SPONSOR,** except to the extent necessary to obtain informed consent from persons receiving the investigational product or their legal guardians, or for discussions with local regulatory authorities, Institutional Review Boards, Ethics Committees, or persons participating in the conduct of the study.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

SPONSOR SIGNATURE PAGE

Protocol Title: A Randomized, Double-Blind, Placebo-Controlled, Study to Evaluate the Safety and Efficacy of LB1148 in Accelerating the Time to Return of Bowel Function in Subjects Undergoing Planned Bowel Resection (INTEGRITY)

Protocol Number: PBI-POI-301

This clinical study protocol was subject to critical review and has been approved by the Sponsor. The following personnel contributed to writing and/or approving this protocol:

[redacted] _____ _____
[redacted]
[redacted]

[redacted] _____ _____
[redacted]
[redacted]

INVESTIGATOR SIGNATURE PAGE

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Protocol Number: PBI-POI-301

I have read the protocol described above. I agree to comply with all applicable regulations and to conduct the study as described in the protocol.

Signed:_____ Date:_____

Print Name:_____

Site Name:_____

SYNOPSIS

Sponsor: Palisade Bio, Inc.

Study Title: A Randomized, Double-Blind, Placebo-Controlled, Study to Evaluate the Safety and Efficacy of LB1148 in Accelerating the Time to Return of Bowel Function in Subjects Undergoing Planned Bowel Resection (INTEGRITY)

Test Product: LB1148

Name of Ingredients: Tranexamic acid (TXA), polyethylene glycol (PEG) 3350, glucose, electrolytes

Study Number: PBI-POI-301 | **Study Phase:** Phase 3

Study Centers: 30-60 US sites

Objectives:

Primary Objective:

The primary objective is to compare the time to gastrointestinal (GI) 2 (GI-2), defined as the time from the end of surgery to the time of recovery of the upper GI tract (toleration of solid food) and the lower GI tract (first bowel movement) following surgery, whichever occurs last, among subjects treated with LB1148 or placebo.

Secondary Objectives:

Compare the following among subjects treated with LB1148 or placebo:

- Time to first bowel movement.
- Hospital length of stay (LOS) Ready
- LOS Discharge Order Written (DOW)
- LOS Actual

Exploratory Objectives:

Compare the following among subjects treated with LB1148 or placebo:

- Time to GI-3
- Incidence of the following events at Day 90:
  - Hospital readmission
  - Post-operative ileus (POI)
- Opiate use by morphine milligram equivalent (MME)

Safety Objectives:

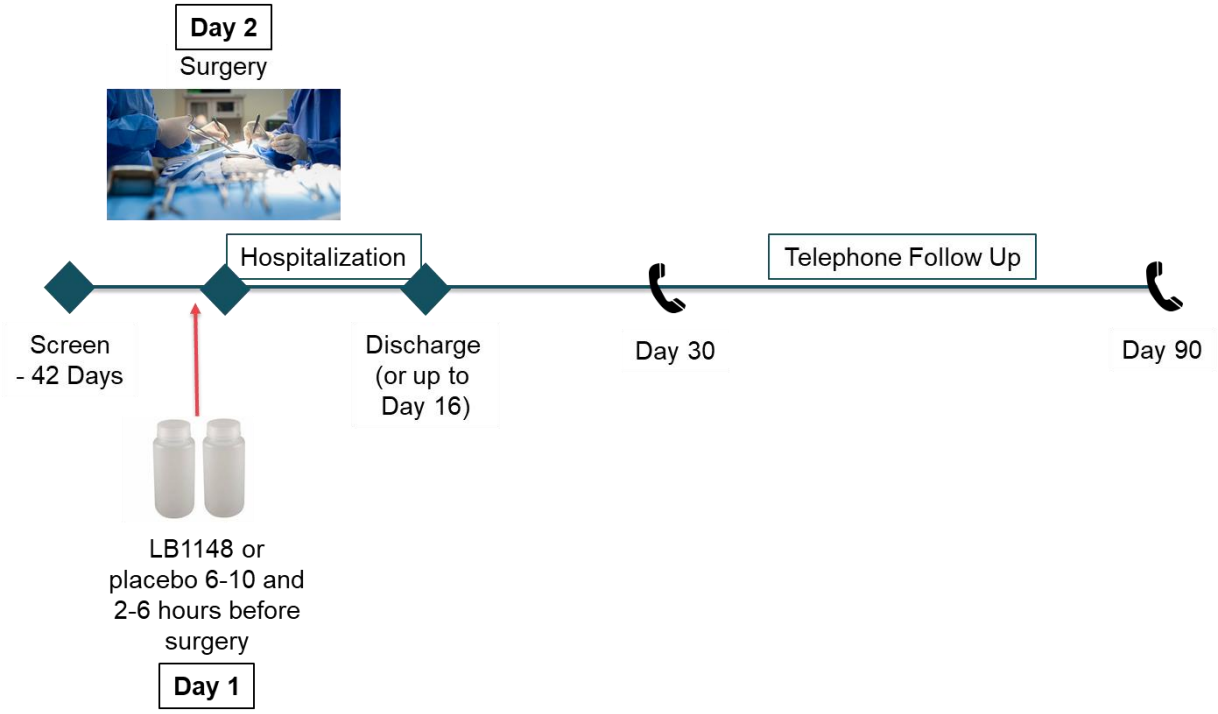
- Evaluate the incidence, severity and potential causal association of treatment emergent adverse events following exposure to LB1148.

Study Design:

This is a Phase 3, randomized, double-blind, placebo-controlled, study to evaluate the safety and efficacy of LB1148 in subjects undergoing planned bowel resection. Subjects scheduled for planned bowel resection, aged 18 to 80 years inclusive, will be screened within 42 days of randomization. Subjects who meet all inclusion and no exclusion criteria, and provide written informed consent, will be stratified by 1) surgical approach (minimally invasive or laparotomy) and 2) with or without a planned stoma. Subjects will then be randomized to receive LB1148 or placebo in a in a 1:1 ratio.

All subjects will receive 700 mL of Investigational Product (IP) as split, oral doses of 350 mL beginning the evening prior to surgery (Day 1), the first at 6-10 hours and the second 2-6 hours prior. Subjects will then undergo surgery (Day 2).

Subjects will be assessed for safety and tolerability, including adverse events (AEs) and vital signs from time of dosing on Study Day 1 through discharge or Study Day 16, whichever is sooner. Subjects will be monitored for return of bowel function following surgery, from Study Day 3 through discharge or Study Day 16, whichever is sooner. Subjects will have clinical lab tests (chemistry, coagulation, and hematology) on Study Day 3. Subjects will be monitored for serious AEs (SAEs) through Study Day 30. Subjects will be monitored for hospital readmission and POI through Study Day 90.



Data Monitoring Committee

An independent DMC will be commissioned for this study. The DMC will be comprised of 3 physicians with expertise in therapeutic areas pertinent to this protocol; all will have clinical trial experience. The DMC safety monitoring plan will be detailed in the DMC Charter.

Number of Subjects Planned: 600

Diagnosis and Main Eligibility Criteria:

Inclusion Criteria

1. Adults age 18 to 80 years, inclusive.
2. Scheduled to undergo a planned (non-emergent) bowel resection via minimally invasive technique or laparotomy. This includes any subject in which a resection of the small intestine, colon, or rectum is performed for any elective indication.
3. Willing to perform and comply with all study procedures including, being hospitalized until achieving GI-2 and responding to telephone follow-up visits as scheduled.
4. Willing and able to provide written informed consent.

Exclusion Criteria

1. History of total colectomy.
2. Has a preexisting ostomy.

3. History of radiation enteritis.
4. Estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m^2.
5. History of seizure disorder.
6. History of myeloproliferative disorders.
7. American Society of Anesthesiologists (ASA) Class IV or V (Appendix 3).
8. Inability to take IP orally or consume solid food.
9. Planned treatment with alvimopan (Entereg®) during hospitalization period.
10. Chronic opioid usage, defined by the American Pain Society as daily or near-daily use of opioids for at least 90 days.
11. Men and women of child bearing potential (WOCBP) who are unwilling to practice a highly effective method of contraception that may include, but is not limited to, abstinence, sex only with persons of the same sex, monogamous relationship with vasectomized partner, vasectomy, hysterectomy, bilateral tubal ligation, licensed hormonal methods (but not combination hormonal methods), intrauterine device, or use of spermicide combined with a barrier method (e.g., condom, diaphragm) for 28 days before Day 1 and through Day 30.
12. Women who will not agree to stop combined hormonal contraceptive and or combined hormonal replacement therapy (including combined hormonal pill, patch or vaginal ring) during the 7 day period prior to Surgery (Study Day 2) through the 7 day period following surgery (Study Days 3 through 9).
13. Has contraindications or potential risk factors to taking TXA. These include subjects with:
    - Known sensitivity to TXA
    - Recent craniotomy (past 30 days)
    - Active cerebrovascular bleed
    - Active thromboembolic disease (such as deep vein thrombosis, pulmonary embolism, cerebral thrombosis, ischemic stroke, or acute coronary syndrome)
    - Acute promyelocytic leukemia taking all-trans retinoic acid for remission induction
14. Receipt of any investigational drug within 28 days or 5 half-lives prior to Day 1.
15. Any other condition that, in the opinion of the Investigator would make the subject unsuitable for the study or unable to comply with the study requirements.

Duration of Treatment: Subjects will receive a split dose of IP over the 10 hours prior to surgery. The total duration for subject participation is approximately 4.5 months (Screening through Day 90).

Test Product; Mode of Administration; and Dose:

A total of 700 mL of LB1148 solution will be administered orally as a split dose (350 mL 6-10 hours before surgery and another 350 mL 2-6 hours before surgery).

LB1148 in sterile water contains 7.5 g (68 mM) TXA, 28 g glucose, 32.5 g PEG 3350, and electrolytes (sodium sulfate anhydrous, sodium bicarbonate, sodium chloride, sodium sulfate anhydrous).

Reference Product; Mode of Administration; and Dose:

A total of 700 mL of placebo solution will be administered orally as a split dose (350 mL 6-10 hours before surgery and another 350 mL 2-6 hours before surgery).

Placebo in sterile water contains 28 g glucose, 32.5 g PEG 3350, and electrolytes (sodium sulfate anhydrous, sodium bicarbonate, sodium chloride, sodium sulfate anhydrous).

Endpoints/Criteria for Evaluation:

Efficacy

Primary Endpoint:

- Time to GI-2, defined as the time from the end of surgery to the time of recovery of the upper GI tract (toleration of solid food) and the lower GI tract (first bowel movement) following surgery, whichever occurs later up to 14 days post-surgery (Study Day 16).
  - End of surgery is defined as the time the last skin staple or suture was placed by the surgeon.
  - Toleration of solid food is defined as the time a subject finished a meal that required chewing and experienced no significant nausea/vomiting for 4 hours after the solid meal.
    - Nausea and vomiting must be severe enough to completely interfere with the subject's ability to tolerate food. This does not include expected post-surgical nausea that does not interfere with solid meal ingestion.

Secondary Endpoints:

- Time in hours from placement of the last skin staple or suture to the time of first bowel movement.
- LOS Ready - Time subject is ready for discharge, defined as the time from the end of surgery to the time the subject is ready for hospital discharge solely based on the recovery of GI function, as determined by the surgeon.
- LOS DOW - Time discharge order written, defined as the time from the end of surgery to the time that the hospital discharge order is written.
- LOS Actual - Time of actual discharge, defined as the time from the end of surgery to the time the subject is actually discharged from the hospital.

Exploratory Endpoints:

- Time to GI-3, defined as the time from the end of surgery to the time of toleration of solid food and either first flatus or bowel movement, whichever occurs later up to 14 days post-surgery (Study Day 16).
- Proportion of subjects not readmitted to the hospital following actual discharge by Study Day 90.
- Proportion of subjects without POI:
  - During the planned hospitalization – POI is defined as the inability to tolerate liquid or solids greater than expected post-operative period, confirmed by imaging studies.
  - Following initial discharge – POI is defined as having clinical symptoms (e.g., abdominal cramps, bloating, nausea, vomiting, constipation, difficulty passing gas, and difficulty tolerating a normal diet) and ileus confirmed by imaging studies.
- Total opiate use by aggregate MME from the end of surgery until Actual discharge or Day 16, whichever is earlier.

Safety:

- AEs and serious AEs (SAEs); clinically significant abnormal physical exam findings and laboratory results will be reported as AEs
- Vital signs
- Serum chemistries, coagulation, and hematology

Statistical Methods:

Sample Size:

A total sample size of 600 subjects, randomized in a 1:1 ratio, provides 90% power to detect a difference in median time to GI-2 between groups of 22 hours, assuming a median time to return of bowel function of 70 hours in the LB1148 group and 92 hours in the placebo group, using a 2-sided log-rank test with a significance level of 0.05.

Enrolling 650 subjects allows for 7.8% of subjects to be excluded from the Per Protocol (PP) population.

Primary Efficacy Analyses:

Time to GI-2 will be a time to event analysis involving the production of non-parametric Kaplan-Meier survival curves for the two treatment groups and their comparison via the stratified log-rank test. As a sensitivity/supportive analysis, a Cox proportional-hazard model (PH) analysis with a main effect of treatment and stratified by surgery type (laparotomy versus minimally invasive approaches) and stoma (stoma versus no stoma) will be performed. Inference between the treatment groups for this analysis will be evaluated using the Wald chi-square test.

Control for Multiplicity

Multiplicity will be controlled through a combination of gate keeping and the Hochberg procedure (Multiple Endpoints in Clinical Trials Guidance for Industry, Draft Guidance 2017).

The primary endpoint will be evaluated a significance level of 0.05. If there is significance then the secondary endpoints will be evaluated, otherwise all secondary endpoints will be considered as exploratory.

If the primary endpoint is significant at 0.05, the four secondary endpoints (time to first bowel, LOS Ready, LOS DOW, LOS Actual) will be considered using the Hochberg procedure. The significance level/α critical values are 0.0125, 0.0167, 0.025, and 0.05.

Using the Hochberg procedure, the p-values for each secondary endpoint will be computed and then ordered from largest to smallest. The largest computed p-value will be compared against the largest critical value. If it does not show significance, then the next largest p-value is compared to the next largest critical value. Testing continues until a p-value for an endpoint is statistically significant when compared to its respective critical value. Once an ordered p-value is significant, that endpoint and all endpoints with p-values smaller than that endpoint are considered to have a statistically significant treatment effect.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Term or Abbreviation	Definition
AE	Adverse event
ADL	Activities of daily living
ASA	American Society of Anesthesiologists
BP	Blood pressure
CTCAE	Common Terminology Criteria for Adverse Events
DMC	Data Monitoring Committee
DOW	Discharge order written
eCRF	Electronic case report form
eGFR	Estimated glomerular filtration rate
GCP	Good Clinical Practice
GI	Gastrointestinal
HDPE	High-density polyethylene
HR	Heart rate
ICF	Informed consent form
ICH	International Conference on Harmonization
IP	Investigational Product
IRB	Institutional Review Board
ITT	Intent-to-Treat
IXRS	Interactive response system used for randomization
LOS	Length of stay
MME	Morphine milligram equivalent
NCI	National Cancer Institute
NG	Nasogastric tube
NPO	Nothing by mouth (nil per os)
NPRS	Numeric Pain Rating Scale
OG	Orogastric tube
PEG	Polyethylene glycol
PH	Proportional-hazard
PK	Pharmacokinetics
POI	Post-operative ileus
PP	Per Protocol
REMS	Risk Evaluation and Mitigation Strategies
RR	Respiratory Rate
SAE	Serious adverse event
T	Temperature
TXA	Tranexamic acid
VTE	Venous thromboembolism
WOCBP	Women of child bearing potential

1 INTRODUCTION

1.1 Return of Bowel Function and Ileus

Gastrointestinal (GI) hypomotility is expected after major surgery. However, for some patients undergoing common abdominal surgeries, there is a delay in return of normal bowel function. This is associated with significant discomfort, nausea, vomiting, and the inability to advance the diet postoperatively. If unresolved, this can predispose patients to nosocomial complications, infections, malnutrition, electrolyte derangements, and poor wound healing.

Palliative treatments such as nasogastric (NG) tube placement and extending observation can be uncomfortable and costly. Possible interventions such as total parenteral nutrition expose patients to additional risk. Postoperative ileus is associated with a prolonged length of ICU and hospital stay, increased resource utilization, and risk for readmission due to GI dysmotility or bowel obstruction (Goldstein 2007). In a retrospective study of colon resections, prolonged postoperative ileus was directly associated with the serious complications of intra-abdominal infections, anastomotic leak and a significantly higher mortality risk (3.7 vs. 0.9%, $p < 0.01$) (Moghadamyeghaneh 2016). Treating the root cause of GI hypomotility caused by abdominal surgery has the potential to lower the risk of morbidity and mortality for the over 6 million patients undergoing abdominal surgeries each year in the US (CDC 2010).

GI surgical intervention (incisions, surgical manipulation, and mesenteric hypoperfusion) leads to a breakdown of the intestinal mucosal barrier, and subsequent translocation of intraluminal proteases into the parenchyma of the intestinal tissues and peritoneum where they initiate autodigestion. Protease activity damages tissues, cell surface receptors, activates immune cells and initiates a pro-inflammatory cascade leading to pathology in the intestine and peritoneum (Chang 2012, Delano 2013, Altshuler 2014).

1.2 Limitations of Current Therapy

The mechanisms affecting post-surgical bowel function and inducing a delay in return of normal bowel function are multifactorial, involving the neurologic, inflammatory, proteolytic, hormonal, cell signaling, anesthetic, and narcotic mechanisms (Goldstein 2007). The single approved therapeutic for acceleration of time to GI recovery following surgery is alvimopan (ENTEREG®), a μ-opioid receptor antagonist that helps mitigate the effects of opioids on GI motility (ENTEREG Prescribing Information 2020). Alvimopan is approved only for surgeries that include partial bowel resection with primary anastomosis, has a black box warning for increased incidence of myocardial infarction, and is only available through a Risk Evaluation and Mitigation Strategies (REMS) program for short-term use. There are no approved therapeutics for the acceleration of return of GI function that arise from any of the other etiologies. There are no directed available therapies for patients who are using nonopioid analgesics during surgery, patients undergoing all abdominal surgeries other than partial bowel resection, and patients with cardiac risks that outweigh the benefits of alvimopan treatment. In a retrospective study of 14,781 patients undergoing elective colorectal surgery at 51 hospitals in Washington State from 2009 to 2013, only 11% of patients received alvimopan (Ehlers 2016). In a retrospective study of patients undergoing colon resection in 2012-2013 after alvimopan was widely available, 12.7% experienced prolonged ileus, indicating that patients still face morbidity and mortality risks with

only a single limited available therapy (Moghadamyeghaneh 2016). Hence, delay in return of bowel function in the post-operative setting remains an important unmet medical need.

1.3 LB1148

LB1148 contains tranexamic acid (TXA), polyethylene glycol (PEG) 3350, glucose, and electrolytes. It is reconstituted in water and administered as a split, oral dose (350 mL 6-10 hours and 2-6 hours prior to surgery). TXA is currently approved for its plasminogen inhibition effects in patients with hemophilia who are undergoing tooth extraction and patients with heavy menstrual bleeding (Dubber 1965) demonstrated that TXA also inhibits trypsin, a key digestive protease, at concentrations 4-fold higher than that required for plasminogen inhibition. In addition to trypsin inhibition, TXA is a broad-spectrum serine protease inhibitor with inhibitory activity against chymotrypsin, elastase, enterokinase, as well as others. This activity appears to be independent of plasminogen inhibition.

Summarized below are nonclinical pharmacology studies, toxicology, and clinical trials of LB1148. More detail can be found in the LB1148 Investigator's Brochure.

1.3.1 Nonclinical Pharmacology Studies

Several studies have been performed to demonstrate the mechanism of action and efficacy of TXA and LB1148. When enterally administered in animal studies, LB1148 inhibits key digestive enzymes in the lumen of the small intestine and helps preserve the gut barrier during acute periods of perturbation, shock, hypoperfusion, and ischemia (Chang 2012, Delano 2013, Altshuler 2014). Oral (enteral) administration of LB1148 allows TXA to inhibit the digestive enzymes in the lumen of the bowel and to halt the digestive enzymes proteolytic activity against the intestine and villi.

1.3.2 Toxicology

TXA, PEG 3350, glucose, and electrolytes are substances approved for use in the United States (US).

Tranexamic Acid

The dose of TXA contained in LB1148 is 7.5 g.

TXA is currently approved for its plasminogen inhibition effects in patients with hemophilia who are undergoing tooth extraction (CYKLOKAPRON® injection, for intravenous use) and patients with heavy menstrual bleeding (LYSTEDA® tablets, for oral use). The approved dose for LYSTEDA is 1.3 g three times a day (3.9 g/day) for a maximum of 5 days (LYSTEDA Prescribing Information). The dose of TXA in LB1148 is 7.5 g, administered as a single split dose of 3.75 g 6-10 hours prior to surgery and again, 2-6 hours prior to surgery.

Single dose and repeat dose studies have been performed in more than 5 species, including rats, dogs, and monkeys. In single dose studies the lethal dose 50 (LD50) ranged from 5 g/kg in dogs (human equivalent dose [HED] of 162 g) in a dog to 12.5 g/kg in mice (HED of 60 g).

Adverse ocular effects were observed in a 9-month toxicology study in dogs. Animals were administered TXA in food at doses of 0, 2, 6, or 12 g/kg/day. These doses are approximately 2, 5, and 6 times, respectively, the recommended human oral dose of 3.9 g/day based on AUC and the same, 2.6, and 3.12 times the dose of TXA in LB1148.

At 6 times the human dose (3.12 times the TXA in LB1148), some dogs developed reversible reddening and gelatinous discharge from the eyes. Ophthalmologic examination revealed reversible changes in the nictitating membrane/ conjunctiva. In some female dogs, the presence of inflammatory exudate over the bulbar conjunctival mucosa was observed. Histopathological examinations did not reveal any retinal alteration. No adverse effects were observed at 5 times the human dose (2.6 times the TXA in LB1148).

In other studies, focal areas of retinal degeneration were observed in cats, dogs and rats following oral or intravenous TXA doses at 6-40 times the recommended usual human dose based on mg/m^2 (actual animal doses between 250-1600 mg/kg/day).

Carcinogenicity – Carcinogenicity studies with TXA in male mice at doses as high as 6 times (5000 mg/kg/day) the recommended human dose of 3.9 g/day showed an increased incidence of leukemia which may have been related to treatment. This is 3.12 times the dose of TXA in LB1148. Female mice were not included in this experiment.

Hyperplasia of the biliary tract and cholangioma and adenocarcinoma of the intrahepatic biliary system have been reported in one strain of rats after dietary administration of doses exceeding the maximum tolerated dose for 22 months. Hyperplastic, but not neoplastic, lesions were reported at lower doses. Subsequent long-term dietary administration studies in a different strain of rat, each with an exposure level equal to the maximum level employed in the earlier experiment, have failed to show such hyperplastic/neoplastic changes in the liver.

Mutagenesis - TXA was neither mutagenic nor clastogenic in the *in vitro* Bacterial Reverse Mutation Assay (Ames test), *in vitro* chromosome aberration test in Chinese hamster cells, and in *in vivo* chromosome aberration tests in mice and rats.

Impairment of Fertility - Reproductive studies performed in mice, rats and rabbits have not revealed any evidence of impaired fertility or adverse effects on the fetus due to TXA.

PEG 3350

The amount of PEG 3350 in LB1148 is 32.5 g total. However, each split dose of LB1148 contains 16.25 g of PEG 3350 which is comparable to the over the counter, osmotic laxative MiraLAX dose, which contains 17g of PEG 3350. Published PK studies of PEG 3350, including MiraLAX, demonstrate that it is minimally absorbed, and is rapidly excreted and primarily eliminated via feces (Pelham 2008).

Glucose and Electrolytes

LB1148 contains 28 g of glucose; about the same as a cup of apple juice.

LB1148 contains electrolytes as follows: sodium sulfate anhydrous - 3.98 g; sodium bicarbonate - 1.18 g; sodium chloride - 1.03 g, and potassium chloride - 0.52 g. These electrolytes are also found in PEG 3350 containing mechanical bowel preparations in amounts 2-4 times higher than those in LB1148.

1.3.3 Clinical Trials of LB1148

Palisade Bio, Inc. (Palisade) and its partner [redacted] have completed or terminated 3 clinical trials of LB1148. One study is ongoing in the US.

LB1148-PK-101

Study LB1148-PK-101 was a Phase 1 single dose, open label study where LB1148 was administered to healthy adults following a 10-hour fast. Blood samples for measuring TXA levels were obtained, just prior to dosing and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48, 72, 96 and 144 hours after dosing.

Among the 12 healthy subjects who participated and completed the trial, 6 were men and 6 were women. The average age was 33.3 years (SD = 7.62). The average weight and BMI were 60.1 kg (SD = 4.68) and 22.4 kg/m2 (SD = 1.35), respectively. The average weights of male and female subjects were 63.2 kg and 57.0 kg, respectively, and the BMI was 22.0 kg/m2 and 22.8 kg/m2 respectively.

PK parameters were calculated and analyzed using Phoenix™ WinNonlin® 8.1 (Pharsight Corp., Mountain View, CA USA).

Table 1 below summarizes the PK parameters of TXA following a single oral dose.

Table 1 Tranexamic Acid Pharmacokinetic Parameters after a Single Oral Dose of LB1148 in Healthy Subjects

	C max	T max	AUC 0-t	AUC 0-∞	%AUC ex	t ½	λ z	CL/F	V Z /F
	(µg/mL)	(h)	(µg ·h/mL)	(µg ·h/mL)	%	(h)	(1/h)	(L/h)	(L)
N	12	12	12	11 *	11 *	11 *	11 *	11 *	11 *
Mean	31.4	2.81	178.57	181.42	2.89	7.9	0.1	44.42	499.16
SD	7.97	0.58	52.07	53.59	1.45	2.43	0.03	11.75	187.77
CV%	25.4	20.52	29.16	29.54	50.03	30.77	27.22	26.46	37.62
Median	29.15	2.98	175.41	158.79	2.63	7.4	0.09	47.23	471.02
Min	21.47	1.98	123.05	127.09	1.27	5.16	0.05	25.7	268.75
Max	46.36	3.98	287.84	291.87	5.86	13.33	0.13	59.01	951.56
GeoMean	30.53	2.76	172.02	174.79	2.59	7.6	0.09	42.91	470.45
GeoSD	1.28	1.24	1.33	1.33	1.64	1.34	1.34	1.33	1.43
GeoCV%	24.8	21.83	28.89	28.78	53.01	29.56	29.56	28.78	36.99

Note: * The R^2 adj of subject 1001 is <0.8 , so the subject was not included in the statistics of pharmacokinetic parameters related to λz .

LBS-POI-201-CN

Study LBS-POI-201-CN, was a randomized, double-blind, placebo-controlled proof-of-concept study designed to evaluate LB1148 to improve the recovery of gastrointestinal function (measured in hours to achieve GI-2) and to reduce post-operative ileus (POI) in subjects undergoing elective bowel resection (PROFILE). This study enrolled 131 subjects from 6 sites in [redacted]

The patient population included subjects aged 18 to 80 years who were scheduled for non-emergent bowel surgery, with or without planned stoma. This included any subject undergoing small bowel, colon or rectal resection for any indication.

Subjects were randomized into 2 treatment groups (LB1148 or placebo) at a ratio of 1:1. Subjects were stratified by: 1) surgical method (minimally invasive or laparotomy), and 2) whether there was a planned stoma. Subjects received a split, oral dose of LB1148 or placebo: 350 mL 6-10 and 350 mL 2-6 hours before surgery. Perioperative care was standardized at all sites per study protocol.

GI function, including POI, was evaluated each day while subjects were hospitalized. While in the hospital subjects were monitored closely for venous thrombosis and surgical complications. Adverse events (AEs) were reported from the time of dosing through discharge or Day 14, whichever came first. Serious AEs (SAEs) including POI and hospital readmission were reported through Day 30.

Subjects who required a second surgery (e.g., takedown of a stoma) were evaluated for the presence, extent and severity of adhesions upon opening of the abdomen for the second surgery.

The primary objective was to evaluate the efficacy of oral LB1148 in improving GI function recovery and reducing POI in subjects undergoing elective bowel resection.

Secondary objectives included:

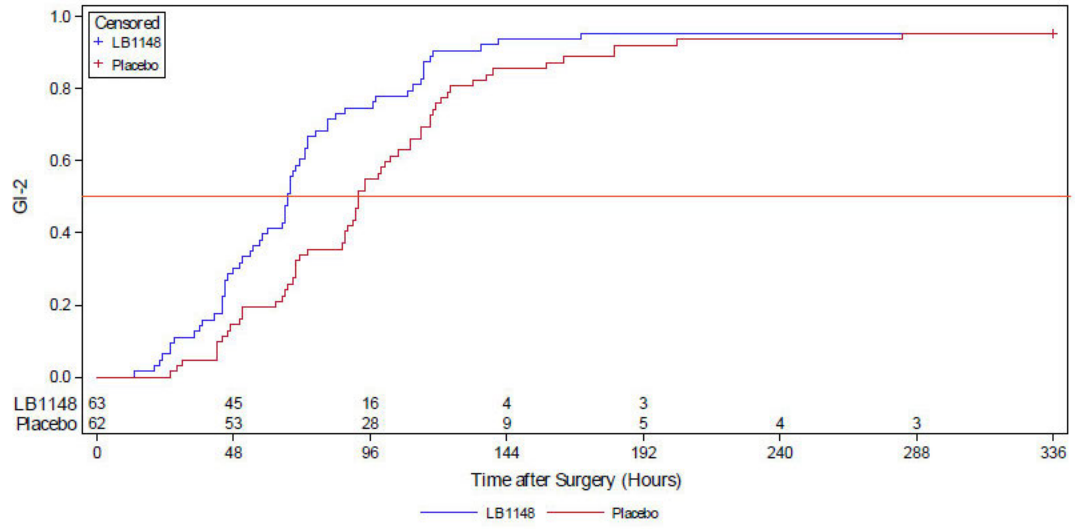
- Safety
- Time to GI-3
- Length of hospital stay (LOS, in hours) to discharge or 14th day (whichever is earlier)
- Presence of intra-abdominal adhesions at time of second surgery (following the initial surgery)

For the LB1148 group, the median and 95% confidence interval of the time to the GI-2 event was 67.0 hours (57.0, 73.0), the mean was 81.4 ± 65.81 hours. Both were lower than the median 92.0 hours (86.0, 106.0) and the mean 107.3 ± 68.88 hours of the placebo group. The median time difference between the two groups was -25.0 hours, that is, the median time from the LB1148 group to the GI-2 event was 25 hours less than the placebo group. The log-rank test results in P values of 0.0015 (with or without stoma stratification) and 0.0098 (without stratification), reaching statistically significant differences. The hazard ratio and the corresponding 95% CI estimated by the stratified (with or without stoma) Cox proportional-hazards (PH) model is 1.800 (1.246, 2.600), and the unstratified value is 1.605 (1.117, 2.307),

indicating that the LB1148 group reached the endpoint event 1.800 times (stratified) or 1.605 times (unstratified) faster than the placebo group.

There was a significant difference in the Kaplan-Meier curve of GI-2 between the two groups (Figure 1), that is, the time required for a GI-2 event to occur in the LB1148 group was shorter than that in the placebo group.

Figure 1 Kaplan-Meier Curve of Time to GI-2



Among subjects dosed there were 46 AEs (71.9%) in the LB1148 group and 50 (79.4%) in the placebo group. Subjects with at least one AE related to the drug was 7 (10.9%) in the LB1148 and 3 (4.8%) in placebo group. A total of 13 subjects had serious adverse events (SAE), including 5 (7.8%) in the LB1148 group and 8 (12.7%) in the placebo group. No SAE related to the drug occurred in the trial, and there was no adverse event that led to drug discontinuation or withdrawal of subjects from the trial. No subject had a venous thrombotic event.

AEs reported in 3 or more subjects receiving LB1148 included: fever (15.6%), nausea (14.1%), hypoalbuminemia (14.1%), vomiting (9.4%), bloating (9.4%), constipation (7.8%), abdominal pain (7.8%), diarrhea (6.3%), decreased blood sugar (6.3%), expectorant cough (6.3%), cough (6.3%), hypotension (6.3%), anemia (4.7%), and increased fibrin D dimer (4.7%).

Overall, AEs are balanced between the groups and there are no apparent trends for type or severity of AEs.

LBS-SS201

LBS-SS201was a Phase 2 study entitled, “Treatment of Septic Shock by Inhibiting Autodigestion and Preserving Gut Integrity with Enteric LB1148 (SSAIL Study).” The study commenced in 2016 and was prematurely terminated the same year due to lack of enrollment.

A total of 8 subjects were enrolled into the study: 5 subjects received LB1148 and 3 received placebo. On average, subjects were exposed to approximately 6 days of study drug (LB1148 or

placebo) with 700 mL administered per day. A total of 3 (60.0%) subjects receiving LB1148 reported 20 AEs and all 3 (100.0%) subjects receiving placebo reported 12 AEs. There were no observed trends for type or severity of AEs. No subject had a venous thrombotic event.

There were 4 SAEs reported in the study, none of which were related to study drug.

LBS-POI-201

LBS-POI-201 is a multicenter, randomized, double-blind, parallel, placebo-controlled, proof-of-concept, adaptive design, Phase 2 study to evaluate LB1148 for return of GI function, reduction of POI and intra-abdominal adhesions in subjects undergoing elective bowel resection with a planned stoma. This study is similar in design to study LBS-POI-201-CN described above. The study is ongoing and to date, 75 subjects have been enrolled.

Among subjects dosed there were 29 AEs (64.4%) in Group A and 25 (83.3%) in Group B. A total of 14 subjects had SAEs none of which were related to the drug, per the investigator. No subject had a venous thrombotic event.

AEs reported in 3 or more subjects included: nausea (28%), vomiting (10.7%), procedural pain (9.3%), urinary retention (5.3%), gastrooesophageal reflux disease (4%), pyrexia (4%), and anaemia (4%).

Overall, AEs are balanced between the groups and there are no apparent trends for type or severity of AEs.

1.4 Rationale for Use of LB1148 for the Return of Bowel Function

As noted above, oral administration of LB1148 allows TXA to inhibit the digestive enzymes in the lumen of the bowel and to halt the digestive enzymes proteolytic activity against the intestine's mucosal barrier, intestinal villi and the peritoneum. In nonclinical models of GI injury, Palisade has demonstrated the ability of LB1148 to protect the intestine from damaging proteolytic activity and thereby enhance the return of bowel function.

Additionally, in a Phase 2 study of LB1148, "A Randomized, Double-Blind, Placebo-Controlled Proof-of-Concept Study Designed to Evaluate LB1148 to Improve the Recovery of Gastrointestinal Function and to Reduce Post-Operative Ileus in Subjects Undergoing Elective Bowel Resection (PROFILE);" conducted in [redacted] subjects receiving LB1148 had a statistically significant 25 hour improvement ($p = 0.0015$) in the return of bowel function following GI surgery compared to subjects receiving placebo.

Nonclinical and clinical findings to date form the rationale for proceeding into this well-powered Phase 3 trial.

1.5 Risk/Benefit Assessment for this Study

More than 100 adult clinical trial subjects have received at least one dose of LB1148. No subject has experienced a SAE thought by the investigator to be related to LB1148. No subject has experienced a venous thrombotic event.

AEs reported in 5 or more clinical trial subjects receiving LYSTEDA (oral TXA) for up to 5 days for the treatment of heavy menstrual bleeding include: headache, nasal and sinus symptoms, back pain, abdominal pain, musculoskeletal pain, arthralgia, muscle cramps and spasms, migraine, anemia, and fatigue (LYSTEDA Prescribing Information).

Warnings and Precautions for the use of LYSTEDA (LYSTEDA Prescribing Information), include:

- Venous and arterial thrombosis or thromboembolism, as well retinal artery and retinal vein occlusions
- Severe allergic reactions
- Cerebral edema and cerebral infarction in patients with subarachnoid hemorrhage
- Ligneous conjunctivitis

The eligibility criteria for this study (Section 4) have been established to limit the risks to subjects, specifically those for whom TXA is contraindicated. Additionally, women are prohibited from using combined hormonal contraception or treatment for 7 days prior to dosing and for 7 days after dosing (Section 5.9.1). During the period of hospitalization (after dosing with LB1148), subjects will be monitored daily for AEs and signs of venous thromboembolism (VTE). The study Medical Monitor will also review safety data continuously throughout the study.

Other than alvimopan, which has limited use in this population (see Section 1.2), there are no approved therapies that accelerate the return of bowel function in subjects undergoing planned bowel resection. Subjects may benefit from shorter time to return of bowel function. Subjects may also benefit from the knowledge that study participation will contribute to the body of knowledge return of bowel function.

The overall benefit-risk assessment for LB1148 is favorable at this time.

2 STUDY OBJECTIVES AND ENDPOINTS

2.1 Study Objectives

2.1.1 Primary Objective

The primary objective is to compare the time to gastrointestinal (GI) 2 (GI-2), defined as the time from the end of surgery to the time of recovery of the upper GI tract (toleration of solid food) and the lower GI tract (first bowel movement) following surgery, whichever occurs last, among subjects treated with LB1148 or placebo.

2.1.2 Secondary Objectives

Compare the following among subjects treated with LB1148 or placebo:

- Time to first bowel movement
- LOS Ready
- LOS DOW
- LOS Actual

2.1.3 Exploratory Objectives

Compare the following among subjects treated with LB1148 or placebo:

- Time to GI-3
- Incidence of the following events at Day 90:
  - Hospital readmission
  - POI
- Opiate use by MME

2.1.4 Safety Objectives

Evaluate the incidence, severity and potential causal association of treatment emergent adverse events following exposure to LB1148.

2.2 Study Endpoints

2.2.1 Primary Endpoint

- Time to GI-2, defined as the time from the end of surgery to the time of recovery of the upper GI tract (toleration of solid food) and the lower GI tract (first bowel movement) following surgery, whichever occurs later up to 14 days post-surgery (Study Day 16).

2.2.2 Secondary Endpoints

- Time in hours from placement of the last skin staple or suture to the time of first bowel movement.
- LOS Ready - Time subject is ready for discharge, defined as the time from the end of surgery to the time the subject is ready for hospital discharge solely based on the recovery of GI function, as determined by the surgeon.
- LOS DOW - Time discharge order written, defined as the time from the end of surgery to the time that the hospital discharge order is written.
- LOS Actual - Time of actual discharge, defined as the time from the end of surgery to the time the subject is actually discharged from the hospital.

2.2.3 Exploratory Endpoints

- Time to GI3, defined as the time from the end of surgery to the time of toleration of solid food and either first flatus or bowel movement, whichever occurs later up to 14 days post-surgery (Study Day 16).
- Proportion of subjects not readmitted to the hospital following initial discharge by Study Day 90.
- Proportion of subjects without POI:
  - During the planned hospitalization – POI is defined as the inability to tolerate liquid or solids greater than expected post-operative period, confirmed by imaging studies. Following initial discharge – POI is defined as having clinical symptoms (e.g., abdominal cramps, bloating, nausea, vomiting, constipation, difficulty passing gas, and difficulty tolerating a normal diet) and ileus confirmed by imaging studies.
- Total opiate use by aggregate MME from the end of surgery until Actual discharge or Day 16, whichever is earlier.

2.2.4 Safety

- AEs and SAEs; clinically significant abnormal physical exam findings and laboratory results will be reported as AEs
- Vital signs
- Serum chemistries, coagulation, and hematology

3 STUDY DESIGN

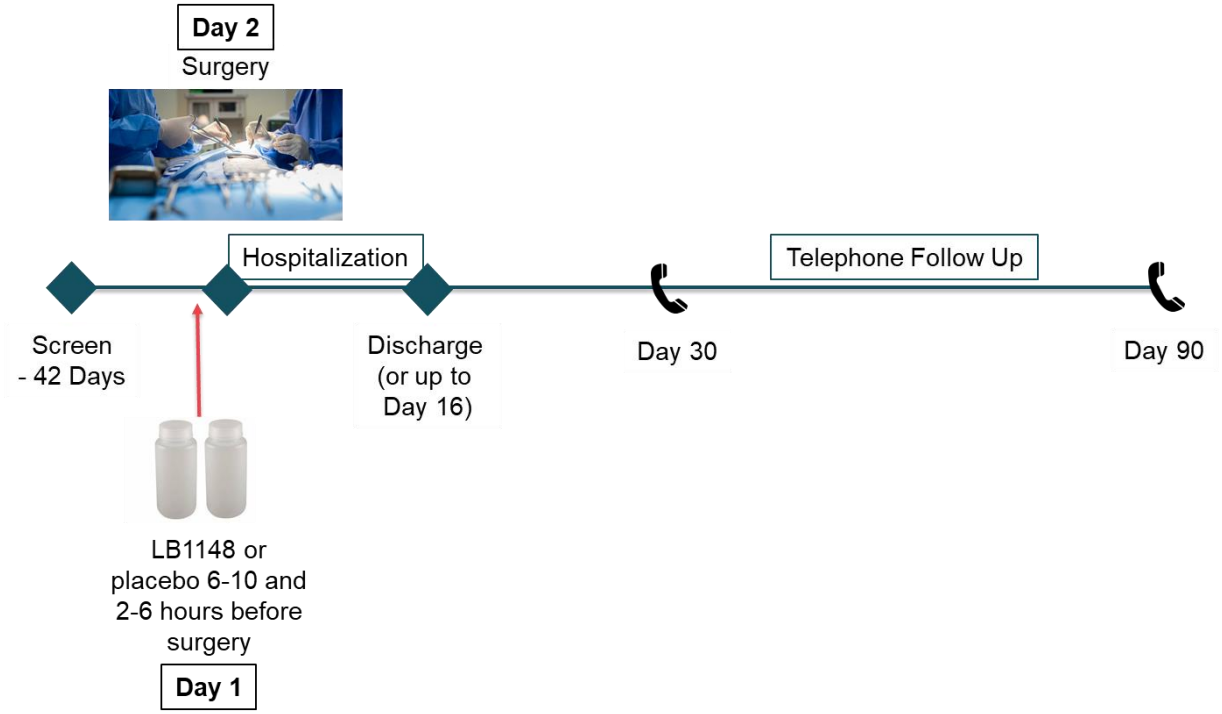
3.1 Overall Study Design

This is a Phase 3, randomized, double-blind, placebo-controlled, study to evaluate the safety and efficacy of LB1148 in subjects undergoing planned bowel resection. Subjects scheduled for planned bowel resection, aged 18 to 80 years inclusive, will be screened within 42 days of randomization. Subjects who meet all inclusion and no exclusion criteria, and provide written informed consent, will be stratified by 1) surgical approach (minimally invasive or laparotomy) and 2) with or without a planned stoma. Subjects will then be randomized to receive LB1148 or placebo in a 1:1 ratio.

All subjects will receive 700 mL of Investigational Product (IP) as split, oral doses of 350 mL beginning the evening prior to surgery (Day 1), the first at 6-10 hours and the second 2-6 hours prior. Subjects will then undergo surgery (Day 2). Subjects will then undergo surgery (Study Day 2).

Subjects will be assessed for safety and tolerability, including AEs and vital signs from time of dosing on Study Day 1 through discharge or Study Day 16, whichever is sooner. Subjects will be monitored for return of bowel function following surgery, from Study Day 3 through discharge or Study Day 16, whichever is sooner. Subjects will have clinical lab tests (chemistry, coagulation, and hematology) on Study Day 3. Subjects will be monitored for serious AEs (SAEs) through Study Day 30. Subjects will be monitored for hospital readmission and POI through Study Day 90.

Figure 2 Study Schematic



The Schedule of Activities is provided in Appendix 1.

3.2 Rationale for Study Design

3.2.1 General Design Issues

This study is intended to be a confirmatory trial to provide evidence that LB1148 is safe and effective in accelerating the time to return of bowel function in subjects undergoing abdominal surgery. Therefore, it is designed as a randomized, double-blind, placebo-controlled, superiority trial.

3.2.2 Selection of Population

The population for this study includes adult patients scheduled to undergo a planned (non-emergent) bowel resection via minimally invasive technique or laparotomy. This includes any subject in which a resection of the small intestine, colon, or rectum is performed for any elected indication. The exclusion criteria include underlying conditions that might put a subject at risk (i.e. where treatment with TXA is contraindicated) or so serious that their inclusion might make either safety or efficacy analyses difficult to interpret.

The study population is the target population for LB1148 and the eligibility criteria allow sufficient homogeneity in terms of co-morbidities to permit precise estimation of treatment effects.

3.2.3 Selection of Dose

As noted in Section 1 of this protocol, TXA inhibits trypsin, a key digestive protease, at concentrations 4-fold higher than that required for plasminogen inhibition (Dubber 1965). In addition to trypsin inhibition, TXA is a broad-spectrum serine protease inhibitor with inhibitory activity against chymotrypsin, elastase, enterokinase, as well as others. Palisade demonstrated that the 90% inhibitory concentration (IC90) was ~68 mM TXA. Nonclinical in vivo studies demonstrated that TXA inhibits the digestive enzymes in the lumen of the bowel and halt the proteolytic activity against the intestine and villi. Concentrations of 68 mM repeatedly demonstrated protection of the bowel in nonclinical animal studies. LB1148 containing 7.5 g of TXA allows for a 68 mM concentration in the intestine.

3.3 Data Monitoring Committee

An independent DMC will be commissioned for this study. The DMC will be comprised of 3 physicians with expertise in therapeutic areas pertinent to this protocol; all will have clinical trial experience. The DMC safety monitoring plan will be detailed in the DMC Charter.

The primary responsibility of the DMC is to safeguard study subjects by reviewing and assessing the clinical safety data being collected during the performance of the study. At scheduled intervals detailed in the DMC charter, the DMC will review all AE data. SAEs (Section 7.2.2) and ≥ Grade 3 AEs that are "Likely" (Section 7.2.3) to be related to IP will be communicated to the DMC in a timely manner, which may result in an *ad hoc* meeting of the DMC.

Based on these evaluations of the data, the DMC will make recommendations to the Sponsor to continue the study as planned, or to modify, temporarily suspend, or terminate the study. The DMC will also be responsible for identifying issues and making recommendations regarding the monitoring of subjects for safety, including collection of additional safety data.

The Sponsor will be responsible for notifying Investigators and Regulatory Authorities of any DMC recommendations, as appropriate.

3.4 Subject Participation and Study Duration

The duration of the study for an individual subject is 90 days, plus up to a 42-day screening window. The estimated duration of the entire study (i.e., first subject screened to last subject off study) is approximately 18 months.

4 SUBJECT POPULATION

Approximately 600 subjects who meet the eligibility criteria will be enrolled at approximately 30-60 sites in the US and multinational.

4.1 Inclusion Criteria

1. Adults age 18 to 80 years, inclusive.
2. Scheduled to undergo a planned (non-emergent) bowel resection via minimally invasive technique or laparotomy. This includes any subject in which a resection of the small intestine, colon, or rectum is performed for any elective indication.
3. Willing to perform and comply with all study procedures including, being hospitalized until achieving GI-2 and responding to telephone follow-up visits as scheduled.
4. Willing and able to provide written informed consent.

4.2 Exclusion Criteria

1. History of total colectomy.
2. Has a preexisting ostomy.
3. History of radiation enteritis.
4. Estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m^2.
5. History of seizure disorder.
6. History of myeloproliferative disorders.
7. American Society of Anesthesiologists (ASA) Class IV or V.
8. Inability to take IP orally or consume solid food.
9. Planned treatment with alvimopan (Entereg®) during hospitalization period.
10. Chronic opioid usage, defined by the American Pain Society as daily or near-daily use of opioids for at least 90 days.
11. Men and women of child bearing potential (WOCBP) who are unwilling to practice a highly effective method of contraception that may include, but is not limited to, abstinence, sex only with persons of the same sex, monogamous relationship with vasectomized partner, vasectomy, hysterectomy, bilateral tubal ligation, licensed hormonal methods (but not combination hormonal methods), intrauterine device, or use of spermicide combined with a barrier method (e.g., condom, diaphragm) for 28 days before Day 1 and through Day 30.
12. Women who will not agree to stop combined hormonal contraceptive and or combined hormonal replacement therapy (including combined hormonal pill, patch or vaginal ring) during the 7 day period prior to Surgery (Study Day 2) through the 7 day period following surgery (Study Days 3 through 9).
13. Has contraindications or potential risk factors to taking TXA. These include subjects with:
    - Known sensitivity to TXA
    - Recent craniotomy (past 30 days)

- Active cerebrovascular bleed
- Active thromboembolic disease (such as deep vein thrombosis, pulmonary embolism, cerebral thrombosis, ischemic stroke, or acute coronary syndrome)
- Acute promyelocytic leukemia taking all-trans retinoic acid for remission induction

14. Receipt of any investigational drug within 28 days or 5 half-lives prior to Day 1.
15. Any other condition that, in the opinion of the Investigator would make the subject unsuitable for the study or unable to comply with the study requirements.

4.3 Subject and Study Discontinuation

4.3.1 Screening Failures

Subjects who sign and date the informed consent form (ICF) but who fail to meet the eligibility criteria are defined as screen failures. The site shall maintain a screening log, that documents the subject's initials, demographics, and reason(s) for screen failure. A copy of the log should be retained in the Investigator's study files.

4.3.2 Premature Discontinuation from Investigational Product

Any subject who does not self-administer the entire contents of both bottles of IP will be considered "prematurely discontinued from IP." The reason for not taking IP as intended by protocol will be reported to the study team and collected in the eCRF. If the reason is due to an AE, it will be reported in accordance with Section 7 - Adverse Events of the protocol (including relatedness and severity).

Subjects who discontinue IP for any reason should remain in the study. All efforts will be made to ensure appropriate follow up, including all relevant evaluations for safety, clinical assessments, and collection of laboratory study results as described in this protocol.

4.3.3 Premature Discontinuation from Study

A subject may be prematurely discontinued from the study for any of the following reasons:

- Subject wishes to withdraw consent
- Subject is lost to follow-up
- Subject is non-compliant or unwilling to comply with the procedures required by the protocol
- Investigator discretion
- Sponsor request

4.3.4 Replacement of Subjects

Subjects who are randomized but do not receive study IP or have the planned surgery will be replaced to ensure the study enrolls sufficient numbers of subjects for the primary analysis.

4.3.5 Study or Site Termination

Conditions may arise during the study that could prompt the study to be halted or the study site to be terminated. Conditions that may prompt such considerations include, but are not limited to, the following:

- The discovery of unexpected, serious, or unacceptable risk to the subjects enrolled in the study.
- A decision on the part of Sponsor to suspend, discontinue, or shorten the study.
- Study conduct at the study site may warrant termination under conditions that include the following:
  - Failure of Investigator(s) to enroll eligible subjects into the study
  - Failure of Investigator(s) to comply with International Conference of Harmonisation-Good Clinical Practice (ICH-GCP) guidelines, or FDA guidelines and regulations
  - Submission of false information from the research facility to the Sponsor, the Clinical Monitor, the FDA, or Institutional Review Board (IRB)
  - Insufficient adherence to protocol requirements
  - A conflict of interest of the Investigator, his/her institution, or site personnel that would negatively impact the integrity of the clinical trial
  - Institution or IRB under investigation for cause by a regulatory agency

5 INVESTIGATIONAL PRODUCT

5.1 LB1148

5.1.1 Description

LB1148 contains TXA, PEG 3350, glucose, and electrolytes. It is formulated in a powder for reconstitution in 640 mL of water (total volume upon reconstitution is 700 mL) and administered as a single split dose. The quantities and concentrations of the individual components of LB1148 are shown in Table 2 below:

Table 2 LB1148 Solution Components by Weight and Concentration

Component	Weight (g)	Concentration (mM)
Tranexamic acid	7.5 g	68.15
Glucose (carbohydrate)†	28 g	222.03
Polyethylene glycol 3350	32.5 g	N/A
Sodium Sulfate Anhydrous	3.98 g	40.06
Sodium Bicarbonate	1.18 g	20.12
Sodium Chloride	1.03 g	25.15
Potassium Chloride	0.52 g	9.83
Water †	640 mL	NA

† packaged separately

5.1.2 Packaging and Labeling

LB1148 powder for reconstitution is packaged in single dose 120 mL high-density polyethylene (HDPE) bottles. Bottles are blinded (contain either LB1148 or placebo) and identified with a "Med #" as shown in Table 3 below.

Table 3 Blinded IP Bottle Label Content

Blinded TXA Active or Placebo Powder For Reconstitution Med #: XXXXXX
RECONSTITUTE AS INSTRUCTED IN PHARMACY MANUAL
Store at controlled room temperature 20°C to 25°C (68°F to 77°F). Excursions permitted between 15°C to 30°C (59°F to 86°F). **DO NOT FREEZE.**
CAUTION: NEW DRUG - LIMITED BY FEDERAL LAW TO INVESTIGATIONAL USE ONLY
Re-labeled by: Thermo Fisher Scientific, 699 N Wheeling Rd, Mount Prospect, 60056
Manufactured for: Palisade Bio, Inc. 5800 Armada Drive, Suite 210, Carlsbad, CA 92008

5.2 Placebo

5.2.1 Description

The placebo for this study is comprised of the same ingredients listed in Table 1 above, except for TXA.

5.2.2 Packaging and Labeling

Placebo powder for reconstitution is packaged in single dose 120 mL HDPE bottles. Bottles are blinded (contain either LB1148 or placebo) and identified with a "Med #" as shown in Table 3 above.

5.3 Glucose Packaging and Labeling

Glucose powder for reconstitution is provided in single dose, open label 60 mL HDPE bottles. The label for the glucose powder is shown in Table 4 below.

Table 4 Glucose Bottle Label Content

Glucose Powder for Reconstitution Lot #: XXXXXX
RECONSTITUTE AS INSTRUCTED IN PHARMACY MANUAL
Store at controlled room temperature 20°C to 25°C (68°F to 77°F). Excursions permitted between 15°C to 30°C (59°F to 86°F). **DO NOT FREEZE.**
CAUTION: NEW DRUG - LIMITED BY FEDERAL LAW TO INVESTIGATIONAL USE ONLY
Re-labeled by: Thermo Fisher Scientific, 699 N Wheeling Rd, Mount Prospect, 60056
Manufactured for: Palisade Bio, Inc. 5800 Armada Drive, Suite 210, Carlsbad, CA 92008

5.4 Storage of Investigational Product

LB1148, Placebo, and glucose should be stored at controlled, room temperature 20°C-25°C (68°F-77°F). Excursions are permitted from 15°C-30°C (59°F-86°F).

5.5 Preparation of Investigational Product for Dispensing to Subjects

Preparation of the IP must be performed by a designated **site pharmacist (or otherwise qualified personnel)** in accordance with the Pharmacy Manual provided by the Sponsor.

Do not freeze powder or reconstituted product. The Sponsor does not have stability data under frozen storage conditions.

5.6 Subject Self-Administration of Investigational Product

Site staff will dispense the subject's IP within 48 hours prior to the subject's scheduled surgery. Subjects will be instructed to consume a bottle of IP at 6-10 hours and the second bottle at 2-6 hours prior to surgery.

5.7 Blinding and Unblinding

Investigators, subjects, and all study staff will be blinded to treatment assignment.

Unblinding of treatment assignment is discouraged. In the event of a medical emergency for which the identity of the treatment assignment is critical to the care of a subject, the Investigator should call the Medical Monitor to discuss. In the event that unblinding is deemed necessary, an unblinded statistician will provide the treatment assignment to the Medical Monitor who will

provide the information to the Investigator. A decision to discontinue a subject from further IP administration is not a rationale to unblind the treatment assignment.

5.8 Concomitant Medications

The following concomitant medications and therapies will be documented in the subject's source document from the morning of Study Day 1 (prior to dosing) through Hospital Discharge or Study Day 16, whichever comes first:

- Prescription and over-the-counter medications, including opioids, bowel prep, and nutritional supplements
- Blood products
- Use of Adhesion Barrier Products including:
  - SEPRAFILM® Adhesion Barrier
  - ADEPT® Adhesion Reduction Solution
  - GYNECARE INTERCEED® Absorbable Adhesion Barrier

5.9 Restrictions

5.9.1 Prohibited Medications

Subjects may not:

- Receive other medication containing TXA from Screening through the 7 day period following surgery (Study Day 9)
- Receive pancreatic enzyme replacement therapy from Day 2 through hospital discharge or Study Day 16, whichever comes first
- Use a combined hormonal contraceptive and or combined hormonal replacement therapy (including combined hormonal pill, patch or vaginal ring) during the 7 day period prior to Surgery (Study Day 2) through the 7 day period following surgery (Study Day 9)
- Use alvimopan (Entereg®) from Day 2 through hospital discharge or Study Day 16, whichever comes first

5.9.2 Perioperative Surgical Preparation

- Subjects may not eat, drink, or take oral medications, including drinking bowel preparations, 1 hour before and 1 hour after IP administration.
- Subjects may not consume solid food within 8 hours prior to induction of surgical anesthesia
- Subjects may not drink a carbohydrate containing drink within 8 hours prior to induction of surgical anesthesia; the IP contains glucose and will replace this drink during this time period
- Subjects may not consume anything by mouth (NPO) within 2 hours prior to induction of surgical anesthesia, except for daily oral medication such as anti-hypertensive may be taken with small volume of water as directed by physician

- Subjects must receive prophylaxis for VTE according to each site's standard of care

5.9.3 Birth Control

Men and WOCBP must be willing to practice a highly effective method of contraception that may include, but is not limited to, abstinence, sex only with persons of the same sex, monogamous relationship with vasectomized partner, vasectomy, hysterectomy, bilateral tubal ligation, licensed hormonal methods (but not combination hormonal methods), intrauterine device or use of spermicide combined with a barrier method (e.g., condom, diaphragm) for 28 days before and after receiving the IP.

5.10 Treatment Compliance

To ensure compliance with the dosing regimen, the IP will be prepared by the site pharmacist or otherwise qualified personnel. Any member of the study team may dispense the blinded, reconstituted dose to the subject or it may be couriered to the subject. Subjects will be instructed to return their used IP bottles for a compliance check prior to surgery on the morning of surgery (Study Day 2).

6 STUDY PROCEDURES

Refer to Appendix 1 for the Schedule of Activities.

6.1 Definitions and Descriptions of Assessments and Procedures

General – results of all of the assessments and procedures described below should be recorded in the subject's electronic medical record or study-specific source document. The following section, Section 6.2 details when these assessments and procedures are to be performed.

AEs and SAEs (see Section 7)

Bowel function – the following information should be collected:

- Time solid food is tolerated (i.e., no significant nausea/vomiting for 4 hours after the solid meal; significant nausea is the inability to tolerate solid food over a 4 hour period)
- nausea/vomiting)
    - Nausea and vomiting must be severe enough to completely interfere with the subject's ability to tolerate food. This does not include expected post-surgical nausea that does not interfere with solid meal ingestion.
- Time of first flatus
- Time of first bowel movement
- POI

  During the planned hospitalization - defined as the inability to tolerate liquid or solids greater than expected post-operative period, confirmed by imaging studies. Resolution of POI, defined as having resolved when all of the following criteria are met:

    - Absence of vomiting for 12 hours without a NG/OG tube
    - Ability to tolerate a solid or liquid oral diet
    - Passage of flatus OR stool over the preceding 24 hours

  Following initial discharge - defined as having clinical symptoms (e.g., abdominal cramps, bloating, nausea, vomiting, constipation, difficulty passing gas, and difficulty tolerating a normal diet) and ileus confirmed by imaging studies

Concomitant Medications (see Section 5.8)

Discharge – the following information should be collected to assess LOS:

- Time discharge is order written
- Time the subject is ready for hospital discharge solely based on the recovery of GI function, as determined by the surgeon ("ready" for discharge)
- The time the subject is actually discharged from the hospital

Laboratory Tests – sites will use their local laboratory for this study

- **Serum chemistries** – chloride, creatinine, glucose, sodium, potassium, carbon dioxide (CO2), blood urea nitrogen (BUN), alanine aminotransferase (ALT), aspartate aminotransferase (AST)**,** bilirubin, and estimation of glomerular filtration rate (eGFR)

- **Hematology** - hemoglobin, hematocrit, red blood cell count, white blood cell count, and platelet count.
- **Coagulation studies -** partial thromboplastin time (PTT), prothrombin time (PT), and International Normalized Ratio (INR)
- **Pregnancy test** – WOCBP must have a serum pregnancy test (serum human chorionic gonadotropin [hCG]) within 14 days prior to randomization and a urine hCG test prior to surgery on the morning of Study Day 2. For this study, WOCBP is defined as not surgically sterile or post-menopausal defined as age > 40 years without menses for ≥ 2 years.
- **Serum** for cytokine and cortisol measurements (see Section 6.3)

Informed consent – written informed consent must be obtained from each subject or legal representative designee (if subject is unable to sign) after the study has been fully explained in accordance with (ICH-GCP). Informed consent must be obtained prior to performing any study-specific procedures. A copy of the signed and dated ICF should be provided to the subject or legal representative, and a copy maintained in the subject's record. The consent process must also be documented in the subject's record.

Medical history, including demographics, GI history and reason for surgery – demographics and a complete medical history of clinically significant diagnoses, including past and present GI diagnoses, and reason for surgery should be obtained from each subject and documented in the medical record. Additionally, the Caprini Risk Factor Score (Appendix 2) will be calculated at the time the Medical History is obtained. Any clinically significant diagnoses occurring from Screening until the subject doses on Study Day 1 should be recorded as Medical History.

Perioperative Surgical Preparation (see Section 5.9.2)

Physical exam:

- **Complete physical exam** – examination of the following systems: cardiovascular, dermatological, ear, nose, and throat, extremities, gastrointestinal, musculoskeletal. ophthalmological, neurological, and respiratory
- **Targeted physical exam** – brief, focused examination of the subject following medical history, and include an assessment of lower extremities for possible VTE, and an assessment for AEs

Surgery information – the following information should be collected:

- Type of operation
- Surgical approach (minimally invasive or laparotomy)
- Location of the incision
- Extent of bowel resection
- Start time and stop time (placement of the last skin staple or suture) of surgery
- Surgical complications should be reported as AEs/SAEs

Telephone Contacts - Every effort must be made to contact the subject or legally authorized representative on Study Days 30 and 90. Documentation of contact and/or attempts at contacting the subject must be recorded in the subject's record.

Vital signs – temperature (T), respiratory rate (RR), heart rate (HR), blood pressure (BP). HR and BP should be obtained after subject is resting for 5 minutes.

6.2 Study Activities by Visit

Each subject who enters Screening will be assigned a subject ID number for traceability. The subject ID will consist of a 3-digit site number and a 3-digit subject number (i.e. 301-001, etc.).

Subjects who sign and date the ICF but who fail to meet the eligibility criteria are defined as Screen Failures. Screen Failure subjects should have their demographic information captured with the reason for screen failure specified.

6.2.1 Screening

The purpose of the screening visit is to determine the subject's trial eligibility by assessing all inclusion/exclusion criteria based on available medical information, clinical assessment, clinical laboratory testing, and subject interview. All subjects will be screened to within 42 days prior to Study Day 1 (dose of IP).

The following assessments and procedures must be performed:

- Obtain written informed consent
- Obtain demographics
- Complete the Caprini risk factor (Appendix 2)
- Complete the ASA Classification (Appendix 3)
- Review medical history, including GI history, reason for surgery, and concomitant medications
- Perform a complete physical exam, including vital signs, height and weight
- Collect laboratory samples for the following:
  - Serum chemistries
  - Hematology
  - Coagulation studies
  - Serum pregnancy test for WOCBP should be collected **within 14 days of randomization**
- Confirm eligibility; note that if it becomes apparent anytime during screening that the subject is not eligible, do not complete additional procedures and document the reason for screen failure

6.2.2 Randomization

Upon determination that a subject meets all eligibility criteria, the subject will be stratified by 1) surgery type (laparotomy versus minimally invasive approaches) and planned stoma (yes or no), then randomized to a specific treatment assignment. Randomization should occur via IXRS within 48 hours of Study Day 1.

6.2.3 Study Day 1 (IP administration)

Blinded IP can be dispensed up to 48 hours prior to surgery. All subjects will take 700 mL of IP as the following split, oral doses:

- 350 mL 6-10 hours prior to surgery, and
- 350 mL 2-6 hours prior to surgery.

Eating, drinking, consuming bowel preparation, and taking oral medications should be held 1 hour before and 1 hour after IP administration.

All subjects will be provided a Dosing Instruction Form that includes date and windows for dosing, and room for the subject to document actual time of dosing and any problems with dosing.

6.2.4 Study Day 2 (Day of Surgery)

The following assessments and procedures must be performed prior to surgery:

- Review concomitant medications including the Dosing Instruction Form to ensure the subject took IP as instructed
- Perform urine pregnancy test for WOCBP
- Perform targeted physical exam, including vital signs
- Obtain blood pre-operatively for cytokine and cortisol measurements (use serum separator tube, spin and mail to the lab the same day. Do not freeze.)
- Assess AEs (or update Medical History in the case of a physical exam finding not previously reported)

Perform surgery and document the appropriate surgical information per Section 6.1.

6.2.5 Study Days 3 through Discharge (or Day 16, whichever is sooner)

- Perform targeted physical exam, including vital signs
- Assess adverse events
- Review concomitant medications
- Complete bowel function assessments per Section 6.1
- On Study Day 3 ONLY, collect laboratory samples for the following:
  - Serum chemistries
  - Hematology
  - Coagulation studies
  - Obtain blood for cytokine and cortisol measurements (use serum separator tube, spin and mail to the lab the same day. Do not freeze.)

- On Study Day 7 ONLY or day of discharge, whichever is sooner, obtain blood for cytokine and cortisol measurements (use serum separator tube, spin and mail to the lab the same day. Do not freeze.)

6.2.6 Hospital Discharge

Subjects should not be discharged until the following criteria are met:

- Passage of stool
- Ability to tolerate solid food and drink liquids

- Perform all activities listed above in Section 6.2.5
- Document discharge information per Section 6.1

6.2.7 Study Day 30 (± 2 days)

Site study staff will contact the subject or their legally authorized representative to determine if the subject experienced POI or any SAE, including hospital readmission.

6.2.8 Study Day 90 / End of Study (± 7 days)

Site study staff will contact the subject or their legally authorized representative to determine if the subject experienced POI or hospital readmission. If readmission did occur, this event should be recorded as a SAE.

Additionally, complete subject disposition form and exit the subject from the study.

6.3 Storage of Research Samples

Approximately 8.5 mL of blood will be collected into an appropriate tube on Study Day 2 prior to surgery, Study Day 3, and Study Day 7/ day of discharge, whichever is sooner. Samples will be processed and stored until shipment to the lab. Stored samples may be used by the Sponsor or its research partners for better understanding of how LB1148 and/or safety analyses. No human genetic testing will be performed on these samples without expressed consent of study subjects. At the conclusion of this study, these samples may be retained in storage by the Sponsor or its research partners for a period up to 15 years.

7 ADVERSE EVENTS

AEs will be reported in a manner consistent with the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) (link provided in Appendix 4).

Adverse events that are expected to occur during this study are as follows:

- Acute Kidney Failure
- Anastomotic leak
- Dehydration
- Intra-abdominal abscess
- Peritonitis
- Post-operative ileus
- Small bowel obstruction
- Urinary Retention

Adverse events listed in the labeling of marketed TXA products (Lysteda™, Cyklokapron™) include:

- Anemia
- Fatigue
- Headache, including migraine
- Muscle cramps
- Pain (back, abdominal, joint, musculoskeletal)
- Sinus and nasal symptoms

Adverse events that have been observed with other TXA products and are therefore of particular concern for the DMC are:

- Ligneous conjunctivitis
- Seizures
- Thromboembolism (including stroke, renal occlusion)

7.1 Reporting Responsibilities

All AEs will be recorded in the eCRF, from Study Day 1 through Hospital Discharge or Day 16, whichever is earlier. SAEs will be reported through Study Day 30. Hospital readmissions will be reported as an SAE through Study Day 90. It is the responsibility of the Investigator or Sub-investigator(s) to perform assessment of all AEs/SAEs. Data describing AEs, including SAEs, will be entered in the subject's medical record and eCRF. In addition, SAEs will be reported to the Sponsor as described in Section 7.6.

Subjects who experience AEs, whether serious or not serious, should receive appropriate treatment and medical supervision as clinically indicated. All AEs must be followed until resolution/stabilization or until a time that is mutually agreed upon between the Medical Monitor and the Investigator.

7.2 Definitions

7.2.1 Adverse Event

An AE is 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 AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IP, whether or not related to the IP.

7.2.2 Serious Adverse Event

An AE or suspected adverse reaction is considered "serious" (SAE) if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death
- Life-threatening
- An AE 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 AE that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious 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.

  Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room (ER) or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

If it is not certain that an event meets the above definitions of an SAE, contact the Medical Monitor to discuss.

7.2.3 Relatedness (Causality)

Causality assessment is required for AEs (and SAEs) that occur during clinical investigations. There is currently no standard international nomenclature to describe the degree of causality or relatedness of an AE with the IP. The following terms will be used during this study:

- **Likely** - Reasons to consider an AE likely related to treatment may include, but are not limited to the following:
  - Timing of the event relative to the administration of the IP

- There is a biologically plausible explanation based on the mechanism of action or mode of delivery of the treatment
- No other explanation is likely

- **Unlikely** - An AE with no temporal association with the IP but rather related to other etiologies such as concomitant medications or conditions, procedures, or subject's known clinical state.

7.2.4 Severity

Severity will be reported in accordance with the NCI-CTCAE (Appendix 4).

If an appropriate listing is not present in this table for an AE, the AE will be graded as follows:

- **Grade 1 Mild** - asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
- **Grade 2 Moderate** - minimal, local or noninvasive intervention indicated; limiting age appropriate instrumental activities of daily living (ADL)
- **Grade 3 Severe** or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
- **Grade 4 Life-threatening** consequences; urgent intervention indicated
- **Grade 5 Death** related to AE

7.3 Clinical Laboratory Abnormalities

Any laboratory abnormality deemed clinically significant by the Investigator at Screening should be reported as medical history. Any new clinically significant laboratory findings following Study Day 1 should be reported as an AE. A clinically significant abnormality is a confirmed abnormality (by repeat test) that is changed sufficiently from Screening so that in the judgment of the Investigator a change in management is warranted. This alteration may include monitoring the laboratory test further, initiating other diagnostic tests or procedures, changing ongoing treatment, or administering new treatment.

Whenever possible, the underlying medical diagnosis (e.g., anemia) should be reported as the AE term. Repeated additional tests and/or other evaluations required to establish the significance and etiology of an abnormal result should be obtained when clinically indicated.

7.4 Physical Exam Abnormalities

Any physical exam abnormality deemed clinically significant by the Investigator at Study Day 1 should be reported as medical history. Any new physical exam abnormality deemed clinically significant by the Investigator during the study should be reported as an AE.

7.5 Pregnancy

All pregnancies that occur –including female partners of male subjects – during the study must be reported to the Sponsor and followed to conclusion. The outcome of each

pregnancy must be reported. The subject should be followed per study protocol. Pregnancy alone is not a reason for premature discontinuation from the study.

Pregnancy alone is not an AE, nor is an induced elective abortion to terminate a pregnancy without medical reason. However, an induced therapeutic abortion to terminate a pregnancy due to complications or medical reasons must be reported as an SAE. The underlying medical diagnosis for this procedure should be reported as the SAE term. A spontaneous abortion is always considered an SAE.

7.6 Reporting of Serious Adverse Events

SAEs must be reported to the Sponsor or designee within 1 business day of becoming aware of the event by entering the data on the AE eCRF. If at the time the Investigator submits an initial SAE report the event has not resolved, the Investigator must provide a follow-up as soon as it resolves (or upon receipt of significant information if the event is still ongoing). All SAEs must be followed until resolution/stabilization or until a time that is mutually agreed upon between the Medical Monitor and the Investigator. Upon checking "serious" on the AE eCRF, a notification will be sent to the Medical Monitor and/or designee. Relevant eCRFs (including Medical History, Concomitant Medications, and Adverse Events) must also be completed to provide supporting documentation for the SAE. If there are additional documents that support the SAE (e.g., clinic or hospital records or procedure reports), they should be uploaded to the AE eCRF.

The Sponsor is responsible for notifying the relevant Regulatory Authorities of certain events. It is the Investigator's responsibility to notify the IRB of all SAEs that occur at his or her site. Investigators will also be notified of all unexpected, serious, IP-related events that occur during the clinical trial. Each site is responsible for notifying its IRB of these additional SAEs.

8 STATISTICAL CONSIDERATIONS

8.1 Sample Size

A total sample size of 600 subjects, randomized in a 1:1 ratio, provides 90% power to detect a difference in median time to GI-2 between groups of 22 hours, assuming a median time to return of bowel function of 70 hours in the LB1148 group and 92 hours in the placebo group, using a 2-sided log-rank test with a significance level of 0.05.

Enrolling 650 subjects will allow for 7.8% of subjects not included in the Per Protocol (PP) population.

8.2 Analysis Populations

Intent-to treat (ITT): The ITT population includes all randomized subjects who receive any amount of IP and have the scheduled surgery. Subjects will be analyzed according to the treatment to which they were randomized to receive. It should be noted that although this analysis population definition technically modifies the strict definition of ITT (i.e., all randomized subjects *without* any other conditions), this analysis population as defined is still consistent with the ITT principle given the context of this clinical trial. All analyses corresponding to the primary endpoint and all secondary endpoints will be formally assessed using the ITT population.

Per Protocol Population (PP): The PP population consists of those subjects in the ITT population who meet all inclusion and no exclusion criteria, complete full IP dose, and have no important protocol deviations (Section 9.10). All analyses corresponding to the primary endpoint and all secondary endpoints will be additionally assessed using the PP population for sensitivity analysis purposes.

Safety Population: All randomized subjects who receive any amount of IP. Subjects will be analyzed according to the treatment that they actually received.

8.3 Analysis Conventions

Planned statistical analyses will be detailed in the Statistical Analysis Plan (SAP). This plan will be finalized prior to locking of the final data set and unblinding of results. The general principles are outlined below. Ad hoc exploratory analyses may be performed in addition to those specified, but no claims or conclusions will be drawn other than hypotheses to be tested in future clinical trials.

In general, descriptive statistics for continuous variables will consist of subject count (n), mean (or geometric mean), median, standard deviation (SD), and range; and descriptive statistics for categorical variables will consist of subject counts and percentages.

No imputation of missing data will be performed except for selected times when dates are present, but times are not. The times to impute are tabled below.

Time	Condition	Imputation
Time of final staple or suture	Date Present/Time absent	1. Time Surgery began 2. 12:00 am
Time of first bowel movement	Date Present/Time absent	11:59 pm
Time first toleration of solid food	Date Present/Time absent	11:59 pm
Time subject is ready for hospital discharge solely based on the recovery of GI function, as determined by the surgeon	Date Present/Time absent	1. TDO 2. TAD 3. 11:59 pm
TDO	Date Present/Time absent	1. TAD 2. 11:59 pm
TAD	Date Present/Time absent	11:59 pm
Time of first flatus	Date Present/Time absent	11:59 pm

Listings will not be imputed.

All efficacy and safety data will be included in data listings and summaries.

8.4 Control for Multiplicity

Multiplicity will be controlled through a combination of gate keeping and the Hochberg procedure (Multiple Endpoints in Clinical Trials Guidance for Industry, Draft Guidance 2017).

The primary endpoint will be evaluated at a significance level of 0.05, if there is significance then the secondary endpoints will be evaluated, otherwise all secondary endpoints will be considered exploratory (Wiens 2003).

If the primary endpoint is significant at 0.05, the four secondary endpoints (time to first bowel movement, LOS Ready, LOS DOW, LOS Actual) will be considered using the Hochberg procedure. The α critical values are 0.0125, 0.0167, 0.025, and 0.05.

Using the Hochberg procedure, the p-values for each secondary endpoint will be computed and then ordered from largest to smallest (Hochberg 1988). The largest computed p-value will be compared against the largest critical value. If it does not show significance, then the next largest p-value is compared to the next largest critical value. Testing continues until a p-value for an endpoint is statistically significant when compared to its respective critical value. Once an ordered p-value is significant, that endpoint and all endpoints with p-values smaller than that endpoint are considered to have a statistically significant treatment effect.

8.5 Subject Disposition

The number of subjects who are randomized, treated, complete the study, and reasons for discontinuation from the study will be summarized in tabular format for the safety population. Subject disposition will also be displayed for the safety population in a subject listing.

8.6 Demographic, Baseline, and Surgical Characteristics

Demographics, baseline, and surgical characteristics will be summarized by treatment group and consist of (but not limited to) the following: age, height, weight, sex, ethnicity/race, GI history, reason for surgery, surgical approach, duration of surgery, stoma or no stoma.

Quantitative variables will be summarized by summary statistics (n, mean, SD, minimum, median, and maximum). Qualitative variables will be summarized as counts and percentages. Demographics and characteristics will be summarized for both the safety population and ITT population.

8.7 Medical History

All medical history (past and current medical disorders), including GI history, will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) version currently in use at the start of the study. Medical history will be summarized by MedDRA System Organ Class (SOC) and Preferred Term, and results presented by treatment group for the safety population.

8.8 Concomitant Medications

All medications will be coded using the World Health Organization (WHO) drug dictionary version currently in use at the start of the study and summarized by treatment group, drug class, and medication term.

8.9 Drug Exposure

The number and percent of subjects who consumed all or part of their IP will be summarized by treatment group for the ITT population.

8.10 Efficacy Analyses

8.10.1 Primary Endpoint - Time to GI-2

Analyses

The primary endpoint is the time to GI-2, defined as the time in hours from the end of surgery to the time of recovery of the upper GI tract (toleration of solid food) and the lower GI tract (first bowel movement) following surgery, whichever occurs later up to 14 days post-surgery.

- End of surgery is defined as the time the last skin staple or suture was placed by the surgeon.
- Toleration of solid food is defined as the time a subject finished a meal that required chewing and experienced no significant nausea/vomiting for 4 hours after the solid meal.

Time to GI-2 will be presented using descriptive statistics by treatment group. Non-parametric Kaplan-Meier survival curves will be produced for the two treatment groups and compared between treatment groups using a log-rank test adjusted with the following stratification factors:

- Type of surgery (laparotomy versus minimally invasive approaches), and

- Stoma construction (Y/N)

In addition to the non-parametric survival curves, a semi-parametric, stratified Cox PH model will be performed for sensitivity analysis purposes, with treatment as the main effect, and surgery type (laparotomy versus minimally invasive) and stoma construction (Y/N) as strata. Comparison of treatment groups and assessment of the significance of the parameter estimates for surgery type and stoma will be made using the Wald chi-square test. Interactions between treatment and the stratification factors will considered as part of the model building process. In order to further investigate the sensitivity of the analysis, a second run of the stratified Cox PH model will add the additional strata "Any opiates" taken after surgery (Y/N).

Ties will be accounted for in the analyses above by selecting the Fleming -Harrington (FH) option in lieu of the default Kaplan Meir estimates. If there are no ties FH estimates are equivalent to Breslow estimates. Likewise, Breslow estimates will be selected for the Cox PH model.

All results will be summarized and listed by treatment group for the ITT and PP populations.

Estimand and Associated Intercurrent Events

The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) E9(R1) guidance [E9-R1_Step4_Guideline_2019_1203.pdf (ich.org)] defines Intercurrent Events (IcE) as "Events occurring after treatment initiation that affect either the interpretation or the existence of the measurements associated with the clinical question of interest." The clinical question of interest addressed here is the estimand associated with the primary endpoint, i.e., the difference in the time to return of normal GI function, as measured by GI-2 (as defined above), between subjects receiving LB1148 and subjects receiving placebo for the treatment of return of bowel function after GI surgery. As described above, this difference will be measured by Kaplan-Meier (log rank test) and its sensitivity ascertained with two Cox PH models (Wald chi square). The timeframe of reference is Study Day 2 through discharge or 14 days after surgery (Study Day 16), whichever is earlier.

The IcEs that may affect the interpretation or the occurrence of this estimand are tabulated below in Table 5, along with the related censoring rules for each analysis.

Table 5 Intercurrent Events for Time to GI-2

	Primary Analysis Approach (Kaplan-Meier and Cox Models)		Further Sensitivity Analyses (Kaplan-Meier Model Only)	
Intercurrent Event	**First Run of Cox Model**	**Second Run of Cox Model**	**#1**	**#2**
No GI-2 but death report	**Censoring at Study Day 16**	**Censoring at Study Day 16**	**Censoring at the date of death**	***Descending Event Dates (Controls)**
No GI-2 and the subject is lost to follow-up or withdrew informed consent	**Censoring at Study Day 16**	**Censoring at Study Day 16**	**Censoring at the date consent withdrawn**	***Descending Event Dates (Controls)**

No GI-2 within 14 days after surgery	**Censoring at Study Day 16**	**Censoring at Study Day 16**	**Censoring at Study Day 16**	***Descending Event Dates (Controls)**
Opiate use	**Not applicable**	**Add strata "any opiates" (Y/N) to the Cox PH model**	**Not applicable**	**Not applicable**

*Descending Event Dates: All missing values for subjects on placebo due to intercurrent events tabled above should be replaced with day of event as date corresponding to Study Day 15 and compute the log rank and Wald Chi square tests as above, then replace with the date corresponding to Study Day 14 unless Study Day 15 was the actual date of the event and hence should be left as Study Day 15 and then compute the log rank and Wald Chi square tests. Likewise for Study Day 12. Treatment subjects with missing data will be censored using the Primary Analysis Approach at Study Day 16

Further details for handing partial dates and times will be provided in the Statistical Analysis Plan (SAP).

8.10.2 Secondary Endpoints

Time to First Bowel Movement

Analysis

The secondary endpoint, time to first bowel movement, is defined as the time from the end of surgery to the time of the first bowel movement. Time to first bowel movement will be analyzed using time to event methods described above for the primary endpoint, time to GI-2. Time to first bowel movement will be presented in summary tables with descriptive statistics for the ITT and PP populations.

Estimand and Associated Intercurrent Events

The clinical question of interest here is the estimand associated with this secondary endpoint, i.e., the difference in the time of first bowel movement, (as defined above), between patients receiving LB1148 and patients receiving placebo for the treatment of return of bowel function after GI surgery. As described above, this difference will be measured by Kaplan-Meier (log rank test) and its sensitivity ascertained with two Cox PH models (Wald chi square). The timeframe of reference is Study Day 2 through discharge or 14 days after surgery (Study Day 16), whichever is earlier.

The IcEs that may affect the interpretation or the occurrence of this estimand are tabulated below in Table 6, along with the related censoring rules for each analysis.

Table 6 Intercurrent Events for Time to First Bowel Movement

	Primary Analysis Approach (Kaplan-Meier and Cox Models)		**Further Sensitivity Analysis (Kaplan-Meier) Model Only)**
Intercurrent Event	**First Run of Cox Model**	**Second Run of Cox Model**	**#1**
No bowel movement but death report	**Censoring at Study Day 16**	**Censoring at Study Day 16**	Censoring at the date of death
No bowel movement and the subject is lost to follow-up or	**Censoring at Study Day 16**	**Censoring at Study Day 16**	Censoring at the date consent withdrawn

Intercurrent Event	Primary Analysis Approach (Kaplan-Meier and Cox Models)		Further Sensitivity Analysis (Kaplan-Meier) Model Only)
	First Run of Cox Model	Second Run of Cox Model	#1
withdrew informed consent			
No bowel movement within 14 days after surgery	**Censoring at Study Day 16**	**Censoring at Study Day 16**	Censoring at Study Day 16
Opiate use	**Not applicable**	**Add strata "any opiates" (Y/N) to the Cox PH model**	Not applicable

Length of Stay – Ready, DOW, and Actual

Analysis

The secondary endpoints, for LOS are defined as follows:

- LOS Ready, is defined as the time from the end of surgery to the time subject is ready for hospital discharge solely based on the recovery of GI function, as determined by the surgeon.
- LOS DOW, defined as the time from the end of surgery to the time that the hospital discharge order is written.
- LOS Actual, is the time of actual discharge, defined as the time from the end of surgery to the time the subject is actually discharged from the hospital.

LOS will be analyzed using time to event methods described above for the primary endpoint, time to GI-2. The second Cox PH model adding "Any opiates" use will be performed only if the strata was significant for the primary endpoint analyses. LOS Ready, DOW, and Actual will be presented in summary tables with descriptive statistics for the ITT and PP populations.

Estimand and Associated Intercurrent Events

The clinical questions of interest here are the estimands associated with these secondary endpoints, i.e., the difference in the time to:

- when the subject is ready for discharge,
- when the discharge order is written, and
- actual discharge,

between patients receiving LB1148 and patients receiving placebo for the treatment of return of bowel function after GI surgery.

As described above, this difference will be measured by Kaplan-Meier (log rank test) and its sensitivity ascertained with at least one Cox PH models (Wald chi square). The timeframe of reference is Study Day 2 through discharge or 14 days after surgery (Study Day 16), whichever is earlier.

The IcEs that may affect the interpretation or the occurrence of these estimands are tabulated below in Table 7, along with the related censoring rules for each analysis.

Table 7 Intercurrent Events for LOS

Intercurrent Event	Primary Analysis Approach (Kaplan-Meier and Cox Models)		Further Sensitivity Analyses (Kaplan-Meier Model Only)
	First Run of Cox Model	Second Run of Cox Model*	#1
Failure to meet discharge criteria (Ready, DOW, Actual) but have a death report	**Censoring at Study Day 16**	**Censoring at Study Day 16**	Censoring at the date of death
Failure to meet discharge criteria (Ready, DOW, Actual) and the subject is lost to follow-up or withdrew informed consent	**Censoring Study Day 16**	**Censoring at Study Day 16**	Censoring at the date consent withdrawn
Failure to meet discharge criteria (Ready, DOW, Actual) within 14 days after surgery	**Censoring at Study Day 16**	**Censoring at Study Day 16**	Censoring at Study Day 16
Opiate use	Not applicable	Add strata "any opiates" (Y/N) to the Cox PH model	Not applicable

*The second Cox PH model adding "Any opiates" use will be performed only if the strata was significant for the primary endpoint analyses.

8.10.3 Exploratory Endpoints

Exploratory endpoints and their analysis methods are described below. All analyses will use the ITT population, and no statistical testing will be formal in nature.

- Time to GI3, defined as the toleration of solid food and either first flatus or bowel movement will be analyzed using time to event methods described above for the primary endpoint, time to GI-2.
- Proportion of subjects not readmitted to the hospital following initial discharge by Study Day 90.
- Proportion of subjects without POI:
  - During the planned hospitalization – POI is defined as the inability to tolerate liquid or solids greater than expected post-operative period, confirmed by imaging studies Resolution of POI, defined as having resolved when all of the following criteria are met:
    - Absence of vomiting for 12 hours without a nasogastric (NG)/orogastric (OG) tube
    - Ability to tolerate a solid or liquid oral diet
    - Passage of flatus OR stool over the preceding 24 hours.
  - Following initial discharge – POI is defined as having clinical symptoms (e.g., abdominal cramps, bloating, nausea, vomiting, constipation, difficulty passing gas, and difficulty tolerating a normal diet) and ileus confirmed by imaging studies.

- Opiate use will be summed using MME. Opiate use will be summarized by treatment group using summary statistics (n, mean, SD, SEM, minimum, first quartile, median, third quartile, and maximum). Comparisons will be made using the Wilcoxon rank sum test.

8.11 Safety Analyses

8.11.1 Adverse Events

All AEs will be coded using the Medical Dictionary for Regulatory Affairs (MedDRA). Frequency tables will be presented by treatment group for all AEs and SAEs by System Organ Class (SOC) and Preferred Term (PT). Frequency tables will also be produced by treatment group for AEs leading to discontinuation from IP and study, by severity, and by causality. No formal statistical testing will be done.

Clinically significant physical exam and lab abnormalities will be reported as AEs and summarized as described above.

8.11.2 Laboratory Evaluations and Vital Signs

Laboratory results and vital signs will be summarized by mean, median, SD, and range. Laboratory abnormalities will be analyzed as safety outcomes by summarizing frequency, severity, and changes from baseline. Other analyses may include but are not limited to the following: examination of shift tables and pre-established severity grades.

9 ETHICAL AND ADMINISTRATIVE RESPONSIBILITIES

9.1 Ethical Conduct of the Study

The procedures set out in this study protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the Sponsor, its authorized US representative, and Investigator abide by good ICH-GCP guideline E6, and in US regulations described in 21 Code of Federal Regulations (CFR) parts 50, 54, 56, and 312. Compliance with these regulations also constitutes compliance with the ethical principles that have their origins in the Declaration of Helsinki.

9.2 Institutional Review Board Approval

This protocol and the ICF and any subsequent modifications will be reviewed and approved by the relevant IRB responsible for oversight of the study. A letter from the IRB indicating approval of the study to be conducted by the Investigator will be provided to the Sponsor prior to initiation of any enrollment at that site. All reviews and approvals by the IRB will be in accordance with 21 CFR part 56.

9.3 Informed Consent

The ICF document must be signed and dated prior to the initiation of study-related tests, and prior to administration of IP. The original signed ICF for each participating subject shall be filed with records kept by the Investigators. A copy of the ICF must be provided to the subject. If applicable, the ICF will be provided in a certified translation of the local language.

9.4 Confidentiality

Personal study subject data collected and processed for the purposes of this study should be managed by the Investigator and his/her staff with adequate precautions to ensure the confidentiality of those data, and in accordance with applicable national and/or local laws and regulations on personal data protection.

Monitors, auditors and other authorized agents of the Sponsor, the IRB approving this research, and any applicable regulatory authorities will be granted direct access to the study subjects' original medical records for verification of clinical trial procedures and/or data, without violating the confidentiality of the subjects, to the extent permitted by the law and regulations. In any presentation of the results of this study at meetings or in publications, the subjects' identity will remain confidential.

9.5 Protocol Amendments

Any changes to the protocol will be made in writing by the Sponsor in the form of a protocol amendment. All protocol amendments will be sent to the Investigator, who is responsible for submitting the amendment to the IRB for approval.

9.6 Case Report Forms

An electronic Case Report Form (eCRF) will be used to record subject data specified by this protocol. The eCRF must be completed by designated and trained study personnel. The eCRF will be signed by the Investigator or a Sub-Investigator listed on the Form FDA 1572. It is the responsibility of the Investigator to ensure the eCRFs are completed and submitted to the Sponsor (or designee) in an accurate and timely manner. The processing of eCRFs will include an audit trail (to include changes made, reason for change, date of change and person making change).

9.7 Source Document Maintenance

Source documents are defined as the results of original observations and activities of a clinical investigation. Source documents may include, but are not limited to, study progress notes, e-mail correspondences, computer printouts, laboratory data, and drug accountability records. All source documents produced in this study will be maintained by the Investigator(s) and made available for inspection by the Sponsor's representatives, the IRB, the FDA, or other regulatory authorities.

9.8 Retention of Records

US regulations (21 CFR part 312.62) require that records and documents pertaining to the conduct of this study and the distribution of investigational drugs including medical records, eCRFs, ICFs, test results, and IP records be kept on file by the Investigator for 2 years after a marketing application is approved for the drug for the indication for which it is being studied. If no application is filed or approved, these records must be kept for 2 years after the investigation has been discontinued and the FDA has been notified. ICH guidelines indicate that documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or at least 2 years have elapsed since the formal discontinuation of clinical development of the IP. No study records should be destroyed without prior authorization from the Sponsor.

9.9 Study Monitoring

Site visits will be conducted by an authorized Sponsor representative (the monitor) to inspect study data, subjects' medical records, and eCRFs in accordance with ICH guidelines, GCPs, and the respective US or national regulations and guidelines, as applicable. It will be the monitor's responsibility to inspect the eCRFs at regular intervals throughout the study, to verify the adherence to the protocol and the completeness, consistency and accuracy of the data being entered. The monitor should have access to laboratory test reports and other subject records needed to verify the entries on the eCRFs.

The Investigator will permit representatives of the Sponsor, the IRB, the FDA, and/or respective health authorities to inspect facilities and records relevant to this study.

9.10 Protocol Deviations

Sites are responsible for abiding by their IRB rules and regulations for reporting protocol deviations. Additionally, the following important protocol deviations will be reported in the eCRF:

- Subject did not meet study eligibility criteria
- Subject did not receive the correct treatment assignment
- Subject did not consume 100% of the assigned dose
- Subject did not have planned surgery
- Subject received a prohibited concomitant medication
- Subject discharged from the hospital prior to achieving GI-2, the primary endpoint

A subject who has 1 of the above deviations will be followed for safety per protocol.

9.11 Financial Disclosure

Investigators participating in this study will provide accurate financial disclosure information to the Sponsor as required by 21 CFR Part 54. Investigators will update the financial information if any relevant changes occur during the study and for 1 year following completion of the study.

9.12 Publication and Disclosure Policy

Investigators and their staff shall hold confidential, and not disclose directly or indirectly to any third party other than those persons involved in the study who have a need to know, the protocol, the data arising out of the study, and any other information related to the study or to Sponsor's products or research programs that is provided to the Investigator. All such persons must be instructed not to further disseminate this information to others. Investigators shall not use the Confidential Information for any purpose other than the study. The foregoing obligations of confidence and non-use assumed by the Investigator shall not apply to: (a) information which at the time of disclosure is in the public domain; (b) information which thereafter lawfully becomes part of the public domain other than disclosure by or through the Investigator; (c) information which, as evidenced by the Investigator's written records, was known by the Investigator prior to the Sponsor's disclosure; (d) information which is lawfully disclosed to the Investigator by a third party not under any obligation of confidence to the Sponsor; or (e) information which is required to be disclosed by law or government regulatory agency, provided reasonable advance notice of such disclosure is given to the Sponsor.

All data and discoveries arising out of the study, patentable or non-patentable, shall be the sole property of the Sponsor. The Sponsor reserves the right of prior review of any publication or presentation of information related to the study. The Sponsor reserves the right of prior review of any publication or presentation of information related to this study. The Sponsor may use these data now or in the future for presentation or publication at the Sponsor's discretion or for submission to government regulatory agencies.

The Sponsor adheres to the general principles of publication of scientific data as articulated by the International Committee of Medical Journal Editors and acknowledges its responsibility to publish results of clinical trials. Persons that fulfill the criteria for authorship (http://www.icmje.org/recommendations/) may be authors on publications based on their contributions to the design, conduct, results, and/or analysis of this clinical trial. Investigators will have access to the data from this clinical trial for the preparation of scientific presentations and publications subject to the requirements of confidentiality. The Sponsor reserves the right to review, within a reasonable time frame, results or analyses from data generated in this study that are intended for public presentation, including scientific meetings.

In signing this protocol, Investigator agrees to the release of the data from this study and acknowledges the above confidentiality and publication policy. The provisions of this Statement shall survive the completion of the study.

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Appendix 1 Schedule of Activities

Study Day/ Assessment	Screening Day -42 to -2	Dosing Day 1	Surgery Day 2	Hospitalization Day 3 to 16/ Discharge [a]	Follow-Up Day 30 (±2 Days)	Follow-Up Day 90 (±7 Days)
Informed consent	X					
Medical history, including diagnosis leading to surgery	X					
Demographics	X					
Confirm eligibility criteria	X	X [b]				
Complete physical exam	X					
Caprini Risk Factor	X					
ASA Classification	X					
Targeted physical exam			X	Daily		
Vital signs	X		X	Daily		
Laboratory Tests				Day 3 only		
Serum chemistries	X			X		
Hematology	X			X		
Coagulation studies	X			X		
Pregnancy test	S [c]		U [c]			
Central lab sample			X [d]	Day 3, Day 7 [e]		
Randomization		X [f]				
Dosing		X [g]				
Bowel function assessment				Daily		
POI assessment				Daily	X	X
Concomitant medication assessment, including opiates	X		X	Daily		
AE, SAE, Hospital Readmission Assessments			X	Daily	SAEs / Hospital Readmission	Hospital Readmission

AE=adverse event, DC=discharge, POI=post-operative ileus, S=serum, U=urine

a. Subjects will be followed in the hospital through Day 16 or discharge, whichever occurs first

b. Eligibility should be confirmed at Screening and just prior to randomization

c. For WOCBP a serum hCG test should be performed 14 days prior to randomization and a urine hCG test prior to surgery on the morning of Study Day 2

d. Prior to surgery

e. Day 7 or day of discharge, whichever comes sooner

f. Randomization and IP preparation should occur within 48 hours prior to dosing

g. Subjects will consume 700 mL of IP in a split dose of 350 mL 6-10 hours prior to surgery and 350 mL 2-6 hours prior to surgery

Appendix 2 American College of Chest Physicians (ACCP) Guidelines - Caprini Risk Factor Score

https://capriniriskscore.org/assessment/

Choose All That Apply

Each Risk Factor Represents 1 Point

- ☐ Age 41-60 years
- ☐ Minor surgery planned
- ☐ History of prior major surgery (<1 month)
- ☐ Varicose veins
- ☐ History of inflammatory bowel disease
- ☐ Swollen legs (current)
- ☐ Obesity (BMI >25)
- ☐ Acute myocardial infarction
- ☐ Congestive heart failure (<1 month)
- ☐ Sepsis (<1 month)
- ☐ Serious lung disease incl. pneumonia (<1 month)
- ☐ Abnormal pulmonary function (COPD)
- ☐ Medical patient currently at bed rest
- ☐ Other risk factors _____

Each Risk Factor Represents 2 Points

- ☐ Age 60-74 years
- ☐ Arthroscopic surgery
- ☐ Malignancy (present or previous)
- ☐ Major surgery (>45 minutes)
- ☐ Laparoscopic surgery (>45 minutes)
- ☐ Patient confined to bed (>72 hours)
- ☐ Immobilizing plaster cast (<1 month)
- ☐ Central venous access

Each Risk Factor Represents 3 Points

- ☐ Age over 75 years
- ☐ History of DVT/PE
- ☐ **Family history of thrombosis***
- ☐ Positive Factor V Leiden
- ☐ Positive Prothrombin 20210A
- ☐ Elevated serum homocysteine
- ☐ Positive lupus anticoagulent
- ☐ Elevated anticardiolipin antibodies
- ☐ Heparin-induced thrombocytopenia (HIT)
- ☐ Other congenital or acquired thrombophilia

If yes:
Type _____

***most frequently missed risk factor**

Each Risk Factor Represents 5 Points

- ☐ Elective major lower extremity arthroplasty
- ☐ Hip, pelvis, or leg fracture (<1 month)
- ☐ Stroke (<1 month)
- ☐ Multiple trauma (<1 month)
- ☐ Acute spinal cord injury (paralysis) (<1 month)

For Women Only (Each Represents 1 Point)

- ☐ Oral contraceptives or hormone replacement therapy
- ☐ Pregnancy or postpartum (<1 month)
- ☐ History of unexplained stillborn infant, recurrent spontaneous abortion (≥3), premature birth with toxemia or growth-restricted infant

Total Risk Factor Score ☐

Abbreviations: BMI = Body Mass Index; COPD = Chronic Obstructive Pulmonary Disease; DVT = Deep Vein Thrombosis; PE = Pulmonary Embolism.

Sources: Caprini 2010, Cronin 2019

Appendix 3 American Society of Anesthesiologists Classification System

Approved by the ASA House of Delegates on October 15, 2014, and last amended on December 13, 2020

Current Definitions and ASA-Approved Examples for Adults

ASA PS Classification	Definition	Examples, Including but not Limited to:
ASA I	A normalhealthy patient	Healthy, non- smoking, no or minimal alcohol use
ASA II	A patient with mild systemic disease	Mild diseases only without substantive functional limitations. Current smoker, social alcohol drinker, pregnancy, obesity (30<BMI<40), well-controlled DM/HTN, mild lung disease
ASA III	A patient with severe systemic disease	Substantive functional limitations; One or more moderate to severe diseases: Poorly controlled DM or HTN, COPD, morbid obesity (BMI ≥40), active hepatitis, alcohol dependence or abuse, implanted pacemaker, moderate reduction of ejection fraction, ESRD undergoing regularly scheduled dialysis, history (>3 months) of MI, CVA, TIA, or CAD/stents.
ASA IV	A patient with severe systemic disease that is a constant threat to life	Recent (<3 months) MI, CVA, TIA or CAD/stents,ongoing cardiac ischemia or severe valve dysfunction, severe reduction of ejection fraction,shock, sepsis, DIC, ARD or ESRD not undergoing regularly scheduled dialysis
ASA V	A moribundpatient who is not expected to survive without the operation	Ruptured abdominal/thoracic aneurysm, massive trauma, intracranial bleed with mass effect, ischemic bowel in the face of significant cardiac pathology or multiple organ/system dysfunction
ASA VI	A declared brain- dead patient whose organs are being removed for donor purposes	

Abbreviations: ARD = advanced renal disease; BMI = body mass index; CAD = coronary artery disease; COPD = chronic obstructive pulmonary disease; CVA = cerebrovascular accident; DIC = disseminated intravascular coagulation; DM= diabetes mellitus; ESRD = end stage renal disease; HTN = hypertension; MI = myocardial infarction; TIA = transient ischemic attack.

Source: ASA website - https://www.asahq.org/standards-and-guidelines/asa-physical-status-classification-system

Appendix 4 National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE)

The following is a link to the full CTCAE, v 5:
https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

Appendix 5 Amendments to the Protocol

Amendment 1, Protocol Version 2.0, March 9, 2022

The overall purpose of this amendment is to:

- Add a Data Monitoring Committee
- Add an exclusion criteria for subjects with severe renal impairment
- Specify when a subject may be discontinued prematurely from IP
- Clarify perioperative surgical preparation restrictions

Effect on Informed Consent Form

- Add perioperative surgical preparation restrictions

Summary of Changes to make v2.0

Section	Change	Rationale
Synopsis – Data Monitoring Committee	Added An independent DMC will be commissioned for this study. The DMC will be comprised of 3 physicians with expertise in hematology, pulmonology or intensive care, and internal medicine; all will have clinical trial experience. The DMC safety monitoring plan will be detailed in the DMC Charter.	FDA recommendation
Section 3.3 – Data Monitoring Committee	Added entire section An independent DMC will be commissioned for this study. The DMC will be comprised of 3 physicians with expertise in hematology, pulmonology or intensive care, and internal medicine; all will have clinical trial experience. The DMC safety monitoring plan will be detailed in the DMC Charter. The primary responsibility of the DMC is to safeguard study subjects by reviewing and assessing the clinical safety data being collected during the performance of the study. The DMC will review SAEs (Section 7.2.2) and ≥ Grade 3 AEs that are “Likely” (Section 7.2.3) to be related to IP as these events may occur. They will also meet	FDA recommendation

Section	Change	Rationale
	periodically throughout the study to review cumulative safety data. Based on these evaluations of the data, the DMC will make recommendations to the Sponsor to continue the study as planned, or to modify, temporarily suspend, or terminate the study. The DMC will also be responsible for identifying issues and making recommendations regarding the monitoring of subjects for safety, including collection of additional safety data. The Sponsor will be responsible for notifying Investigators and Regulatory Authorities of any DMC recommendations, as appropriate.	
Synopsis and Section 4.2 - Exclusion Criteria	Added Exclusion Criteria: 5. Estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m2	FDA request
Section 4.3.2 – Premature Discontinuation from Investigational Product	Added entire section Any subject who does not self-administer the entire contents of both bottles of IP will be considered "prematurely discontinued from IP." The reason for not taking IP as intended by protocol will be reported to the study team and collected in the eCRF. If the reason is due to an AE, it will be reported in accordance with Section 7 - Adverse Events of the protocol (including relatedness and severity). All efforts will be made to follow subjects who discontinue IP for any reason. Such follow up will include all relevant evaluations for safety including clinical assessments and collection of laboratory study results as set out in this protocol.	FDA request
Section 5.9.2 – Perioperative Surgical Preparation	Modified first bullet • Subjects may not eat, drink, **or take oral medications**, including drinking bowel preparations, 1 hour before	FDA request

Section	Change	Rationale
	and 1 hour after IP administration.	
Section 6.1 – Laboratory Tests	Added eGFR to be included with the chemistry panel	FDA request
Section 6.2.3 – Study Day 1 (IP Administration)	Modified second paragraph Eating, drinking, consuming bowel preparation, and **taking oral medications** should be held 1 hour before and 1 hour after IP administration.	FDA request

Amendment 2, Protocol Version 3.0, May 20, 2022

The overall purpose of this amendment is to improve the clarity of the protocol and to add specific information regarding a blood draw for research.

Summary of Changes to make v3.0

Section	Change	Rationale
Administrative	Corrected typos, grammatical errors and errors in references, updated version number and date, updated this table	
Synopsis – Study Centers	Removed and multinational	This study will be conducted only at US sites
Synopsis – Secondary Objectives	Definitions were removed, as they are provided in the endpoints Safety was moved to a separate objective	Safety is listed separately in the statistics section
Synopsis – Secondary Objectives	Definitions were removed, as they are provided in the endpoints	
Synopsis – Study Design	The timing of dosing was clarified	As written it could be inferred that the entire dose needed to be completed on Day 1
Synopsis – I/E Criteria	• Willingness to forgo alvimopam treatment was moved to an affirmative choice, rather than an exclusion criterion • Exclusion regarding emergency surgery was deleted, as it is covered by inclusion criterion #2 • "or current" deleted from exclusion criteria as redundant to "history of"	• This should be a choice made by the subject in consultation with their physician • Redundant • Timeframe of "history" is not defined and therefore includes current time

	• Exc#10 added missing text	• typo
Synopsis – Test Product	added "solution", qualified water as "sterile" for both IPs	To distinguish drug product from reconstituted product
Synopsis –Endpoints	Reworded	For clarity
Synopsis – Data Monitoring Committee	Edited An independent DMC will be commissioned for this study. The DMC will be comprised of 3 physicians with expertise in *therapeutic areas pertinent to this protocol* ~~hematology, pulmonology or intensive care, and internal medicine~~; all will have clinical trial experience. The DMC safety monitoring plan will be detailed in the DMC Charter.	DMC members may have alternate backgrounds
Body of the protocol	All changes to the synopsis are also made in the body of the protocol	
Section 1.3.3 – LBS-POI-201-CN	Added italicized text: "…to improve the recovery of gastrointestinal function *(measured in hours to achieve GI-2)* and…"	for clarity
Section 3.2.2 – Selection of Population	added italicized text: "…sufficient homogeneity *in terms of co-morbidities* to permit	To avoid implying that race, gender or ethnicity will be homogeneous by design
Section 3.3 – Data Monitoring Committee	Edited An independent DMC will be commissioned for this study. The DMC will be comprised of 3 physicians with expertise in *therapeutic areas pertinent to this protocol* ~~hematology, pulmonology or intensive care, and internal medicine~~; all will have clinical trial experience. The DMC safety monitoring plan will be detailed in the DMC Charter. The primary responsibility of the DMC is to safeguard study subjects by reviewing and assessing the clinical safety data being collected during the performance of the study. *At scheduled intervals detailed in the DMC charter, the DMC will review all AE data. SAEs (Section 7.2.2) and ≥ Grade 3*	FDA recommendation

	AEs that are "Likely" (Section 7.2.3) to be related to IP will be communicated to the DMC in a timely manner, which may result in an ad hoc meeting of the DMC. ~~The DMC will review SAEs (Section 7.2.2) and ≥ Grade 3 AEs that are "Likely" (Section 7.2.3) to be related to IP as these events may occur. They will also meet periodically throughout the study to review cumulative safety data.~~	
Synopsis and Section 4.2 - Exclusion Criteria	Renumbered Exclusion Criterion 5 to be 4: 4. Estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m2	Administrative
Section 4.3.2 – Premature Discontinuation from Investigational Product	Edited this section Any subject who does not self-administer the entire contents of both bottles of IP will be considered "prematurely discontinued from IP." The reason for not taking IP as intended by protocol will be reported to the study team and collected in the eCRF. If the reason is due to an AE, it will be reported in accordance with Section 7 - Adverse Events of the protocol (including relatedness and severity). *Subjects who discontinue IP for any reason should remain in the study. All efforts will be made to ensure appropriate follow up, including all relevant evaluations for safety, clinical assessments, and collection of laboratory study results as described in this protocol.* ~~All efforts will be made to follow subjects who discontinue IP for any reason. Such follow up will include all relevant evaluations for safety including clinical assessments and collection of laboratory study results as set out in this protocol.~~	Improved clarity
Section 5.1.1 - Description	Added "solution" to table description and highlighted the components that are packaged separately	For added clarity – LB1148 drug product is not itself a solution

Section 6.1 – Laboratory Tests	Changed "future research" to "Serum for cytokine and cortisol measurements" Added "respiratory rate (RR)" to vital signs	A decision was made to measure inflammatory cytokines and serum cortisol
Section 6.2.1 – Screening	Revised the order of procedures to have simple, potentially disqualifying gradings precede more laborious tasks Removed "Serum for future research"	The timing of the serum collection was adjusted to match an ERAS study
Section 6.2.4 – Study Day 2	Revised the serum collection instructions Added "in the case of a physical exam finding not previously reported"	Addition explains how a potential AE can become medical history
Section 6.2.5 – Study Day 3	Added Clarification regarding serum collection for cytokine and cortisol analysis	
Section 6.3- Storage of Research Samples	Edits made to days of collection, volume and handling	
Section 7 – Adverse Events	Added A listing of adverse events that can be expected in the patient population or have been seen with TXA according to existing drug labels	To avoid declaring adverse events as unexpected
Section 8.3	Added: "No imputation of missing data will be performed except for selected times when dates are present, but times are not. The times to impute are tabled below." The table was added. Added: "Listings will not be imputed."	
Sections 8.7 & 8.8	Clarified that coding of AEs and concomitant medications will be done using the version of MedDRA and WHO Drug	To avoid requiring recoding each time the version changes

	currently in use at the start of the study	
Section 8.10.1 – Analyses	Added "in hours" Added: "Interactions between treatment and the stratification factors will considered as part of the model building process." Added: "Ties will be accounted for in the analyses above by selecting the Fleming -Harrington (FH) option in lieu of the default Kaplan Meir estimates. If there are no ties FH estimates are equivalent to Breslow estimates. Likewise, Breslow estimates will be selected for the Cox PH model." Tables 5, 6 and 7 were revised for clarity.	To define the unit of time For clarification
Appendix 1 – Schedule of Activities	Changed the heading from Assessments to Activities Updated collection times for the research blood sample	Not all activities are assessments The sample will be for cytokine and cortisol measurements

Amendment 3, Protocol Version 4.0, June 7, 2022

The overall purpose of this amendment is to improve the clarity of the protocol.

Summary of Changes to make v3.0

Section	Change	Rationale
Synopsis – I/E Criteria	• Willingness to forgo alvimopam treatment was moved back to an exclusion criterion • Men and women of child bearing potential (WOCBP) who are unwilling to practice a highly effective method of contraception that may include, but is not limited to, abstinence, sex only with persons of the same sex, monogamous relationship with vasectomized partner, vasectomy, hysterectomy, bilateral tubal ligation,	• This should be a choice made by the physician with subject agreement to forgo alvimopam treatment • Moved to exclusion criterion if the subject is unwilling to practice a highly effective method of contraception from Day 1 through Day 30

	licensed hormonal methods (but not combination hormonal methods), intrauterine device, or use of spermicide combined with a barrier method (e.g., condom, diaphragm) for 28 days before Day 1 and through Day 30 moved to an exclusion criterion. • Updated to specify 'women' who will not agree to stop combined hormonal contraceptive and or combined hormonal replacement therapy	• For clarification
Synopsis –Endpoints	Reworded	For clarity
Body of the protocol	All changes to the synopsis were also made in the body of the protocol	
Synopsis and Section 4.2 - Exclusion Criteria	• Added back 'willingness to forgo alvimopam treatment' as an exclusionary criterion • Moved and updated wording that men and women of child bearing potential unwilling to practice a highly effective method of contraception would be excluded • Updated exclusion 12 to specify women who will not agree to stop combined hormonal contraceptive and or combined hormonal replacement therapy would be excluded	Administrative updates per changes made to the synopsis