STATISTICAL ANALYSIS PLAN (SAP)

Protocol Number:	LBS-POI-201
Protocol Title:	A Randomized, Double-Blind, Placebo-Controlled Proof of Concept Study to Evaluate LB1148 for Return of Gastrointestinal Function, Post-Operative Ileus and Intra-Abdominal Adhesions in Subjects Undergoing Elective Bowel Resection (PROFILE)
Product Name or Number:	LB1148
Sponsor:	Palisade Bio, Inc. 5800 Armada Drive, Suite 210 Carlsbad, CA 92008
SAP Prepared by:	[redacted] Innovative Analytics, Inc. 161 East Michigan Ave. Kalamazoo, MI 49007 USA
SAP Version Number (Date):	Version 3.0 (March 10, 2023)
Protocol Version Number (Date)	Amendment #6 (Aug 26, 2022)

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1 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Term	Definition
AE	adverse event
BMI	body mass index
BP	blood pressure
CDC	Centers for Disease Control
CMS	Centers for Medicare & Medicaid Services
COVID-19	coronavirus disease 2019
GI	gastrointestinal
HR	heart rate
IV	intravenous
LBS	Leading BioSciences (previous Sponsor name)
LB1148	investigational product - the active ingredient, TXA, is formulated in a solution containing PEG, glucose, electrolytes, and water
LOS	length of stay
mcg	microgram
mg	milligram
mITT	modified intent-to-treat
MME	Morphine milligram equivalent
NG	nasogastric tube
NPRS	Numeric Pain Rating Scale
OG	orogastric tube
PAI	Peritoneal Adhesion Index
PD	protocol deviation
PEG	polyethylene glycol
PH	proportional hazards
PBI	Palisade Bio, Inc. (Sponsor)
POI	post-operative ileus
PP	per protocol
PV	protocol violation
SAE	serious adverse event
SD	standard deviation
SOP	standard operating procedure
TXA	tranexamic acid - the active ingredient in LB1148
VRS	verbal rating score
VTE	venous thromboembolism

Abbreviation or Term	Definition
WHO	World Health Organization

2 STUDY OVERVIEW

This 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. The study is randomized to control for factors known and unknown between the treatment groups, and neither the Investigator nor the subject will know which treatment the subject will receive in order to avoid any bias in the study assessments.

This study is composed of 5 periods: Screening, Surgical, Post-Surgical, Follow-Up, and Second Surgery.

Subjects scheduled for elective bowel resection, aged 18 to 80 years inclusive, will be screened within 42 days of randomization (Study Day 1). Subjects who meet all inclusion and no exclusion criteria, and provide written informed consent, will be stratified by: 1) surgical approach (either minimally invasive technique or laparotomy) and 2) with or without a planned stoma, and then randomized to receive a split oral liquid dose of LB1148 or placebo (polyethylene glycol [PEG], glucose, and electrolytes) in a in a 1:1 ratio. (Note: Stratification factor #2 does not apply for subjects randomized after 01Jan2022 based on Protocol Amendment #5 updates because all subjects should have a stoma.)

All subjects will receive 350 mL of study drug (test article) 6-10 and 2-6 hours prior to surgery (Study Day 1/2). Subjects will then undergo the first surgery. Prior to surgical closure, the investigator will determine the incidence, extent, and severity of adhesions using the Intra-Abdominal Adhesion Extent and Severity Assessment Worksheet (Study Protocol, Appendix 5).

Subjects will be assessed for safety and tolerability, including AEs, physical exam, vital signs, clinical lab tests (chemistry, coagulation, and hematology) while in the hospital and on Day 30. Subjects will be monitored for return of bowel function while in the hospital and discharged following tolerance of solid food and first bowel movement.

Within 8 months of enrollment, subjects will undergo a second surgery for the purpose of ostomy takedown or other planned abdominal surgery, at which time the surgeon will determine the incidence, extent, and severity of adhesions. If a subject is not ready for the second surgery by 8 months, the investigator may consult with Medical Monitor to obtain window approval.

Unless required for safety reasons or protocol compliance, all subjects, Investigators, and study site personnel will remain blinded to the identity of the treatment from time of randomization until final database lock.

The Schedule of Assessments is provided in Appendix 1 of the protocol.

The study will evaluate the extent and severity of adhesions, as provided in the primary objective (Section 3.1), in order to determine the overall test article effect. Additionally, an evaluation of the secondary (Section 3.2) and exploratory objectives (Section 3.3) related to return of GI function, pain, and POI will be performed.

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective of this study is to compare the change from baseline in extent and severity of intra-abdominal adhesions among subjects treated with LB1148 or placebo.

3.2 Secondary Objectives

The secondary objectives of this study are to determine the following among subjects treated with LB1148 or placebo:

- Compare time from surgical closure to resolution or appearance, as appropriate, of 1 or more of the components common to gastrointestinal (GI) dysfunction following elective bowel resection with a planned stoma.
    - Number of hours to GI2 (see Section 11.2.1 for definition)
    - Number of hours to GI3 (see Section 11.2.1 for definition)
    - Number of hours to resolution of POI
- Compare hospital length of stay (LOS, recorded in hours) through Discharge or Day 14 (whichever comes first).
- Compare the incidence of intra-abdominal adhesions at the opening of the second surgery (operationally this occurs at the time of opening)
- Compare the extent and severity of intra-abdominal adhesions at the opening the second surgery, (operationally this occurs at the time of opening)
- Compare the change from baseline in incidence, extent, and severity of intra-abdominal adhesions among subjects who had adhesions observed at the time of the first surgery
- An additional objective of this study is the evaluation of the safety and tolerability of LB1148 in subjects undergoing elective bowel resection.

3.3 Exploratory Objectives

The exploratory objectives of this study are to determine if LB1148 reduces the:

- Post-operative abdominal pain (first surgery only)
- Incidence of bowel obstruction through 30 days post-operatively (first surgery only)
- Incidence of hospital re-admittance through 30 days post-operatively (first surgery only)
- Volume of blood product transfusions needed on the day of surgery (first surgery only)

4 GENERAL METHODS

4.1 Analysis Populations

Due to coronavirus disease 2019 (COVID-19), some subjects enrolled and randomized prior to 01Jan2022 were released from the hospital prior to achieving GI2. In addition, subjects were randomized based on whether the surgery was to include a stoma or not and a subject was considered completed at Study Day 30, e.g., the second surgery was optional. Protocol Amendment 5 and 6 require subjects to have a stoma during the first surgery and require the second surgery for the subjects to be considered complete. The primary endpoint is now comparing the change from baseline in extent and severity of intra-abdominal adhesions rather than GI2 among subjects treated with LB1148 or placebo. These circumstances and changes require corresponding updates to the populations defined in the Study Protocol.

The **modified Intent-to-Treat (mITT)** population includes all randomized subjects who received any investigational test article on or after 01Jan2022, where subjects will be analyzed according to the treatment to which they were randomized.

Safety Population: The safety population will be defined as all subjects who receive any amount of investigational test article. Subjects will be analyzed according to the treatment actually received.

The **Per Protocol Population (PP)** includes subjects in the mITT population who met all inclusion and no exclusion criteria, received the complete appropriate study dose, and had no important protocol deviations or violations, where subjects will be analyzed according to the treatment to which they were randomized.

Study subjects may be replaced if found to be ineligible (violation of one or more inclusion/exclusion criteria), or if following randomization, did not go on to receive ANY amount of investigational test article.

4.2 Summarization of Data

Study results will be summarized in tabular format by treatment group, with descriptive statistics and/or in subject listings. In general, descriptive statistics for continuous variables will consist of subject count (n), mean, standard deviation (SD), minimum, first quartile, median, third quartile and maximum. Descriptive statistics for categorical variables will consist of subject counts and percentages.

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

The efficacy analyses described in this Statistical Analysis Plan will be performed for the mITT (all efficacy endpoints) and PP Populations (primary efficacy endpoint and selected secondary efficacy endpoints), while the safety analyses will be performed for the Safety Population. All statistical tests will be 2-sided, with p-values considered nominal for this proof-of-concept study, although a p-value <0.05 will be highlighted. Unless otherwise specified, p-values will be reported to four (4) digits. No multiplicity adjustments will be performed.

4.3 Sample Size Justification and Randomization

This proof-of-concept study was originally designed to utilize an adaptive sample size re-estimation to allow for sample size adjustment based on observed data from the interim analysis. No formal knowledge of the effect size is given; therefore, no power calculations were used to assess type I and type II error rates. The goal of this study is to assess the observed effect size at the interim and adjust sample size accordingly.

Initially, subjects were randomized into an active or placebo group following a 1:1 ratio while stratified for surgery type (laparotomy or minimally invasive) and stoma (planned or unplanned). After the first 50-60 subjects were enrolled, an interim analysis was planned to assess GI2 and LOS (Discharge Order Written) endpoints. If the treatment effect size, conditional power, or reproducibility probability was deemed insufficient at the interim, then the sample size would be increased accordingly, from 120 up to 200 subjects.

The sample size justification as originally designed no longer applies to the new primary endpoint (change from baseline in extent and severity of intra-abdominal adhesions among subjects treated with LB1148 or placebo). Enrolling up to 40 subjects is estimated to provide at least 30 randomized subjects, which should be adequate to explore the feasibility of the new endpoint. The data collected from the subjects in this study will be available to power a future study using this endpoint.

Subjects randomized after 01Jan2022 were randomized by surgery type but within the planned stoma arm only.

4.4 Data Handling

4.4.1 Method for Handling Missing Data

No imputation of missing data will be performed except for times when dates are present in “time to” analyses. In those occasions, the latest or earliest time in that day will be imputed (11:59 pm or 12:01 am) to obtain the worst case (longest) for the “time to” being analyzed. (i.e., longest when the endpoint is favorable and shortest when the endpoint is unfavorable.) Time fields in the listings will not include the imputation, however, the computed “time to” fields will.

4.4.2 Day

Start Date and End Date fields in the listings have a Day associated with them. Day = 1 will be determined by the date/time of when the first split dose of the test article is consumed. Days prior to this will be negative.

In the tables and listings when referring to a specific Visit, e.g., Day 0, Day 1,…, ,Day 14, refer to Visit names given in Appendix 1 Schedule of Assessments in the study protocol.

4.4.3 Definition of Baseline Values

Unless otherwise specified, Baseline values are defined as the last values collected before the first split dose of test article.

4.4.4 Windowing of Visits

All data will be categorized based on the scheduled visit at which it was collected. These visit designators are pre-defined values indicated in the Schedule of Assessments (Appendix 1 of the protocol).

4.4.5 Justification of Pooling

Due to the expected low enrollment of subjects at each site, and the multitude of selected sites, data from all sites will be pooled. Study center or treatment-by-center interaction terms will not be included in statistical analyses.

4.5 Output Production and Validation

All analyses will be performed using SAS V 9.3 or higher (SAS Institute, Inc., Cary, North Carolina, USA). Validation and quality control of the tables, listings, and figures which display the results of the statistical analysis of the data from this study, will follow the appropriate Innovative Analytics standard operating procedures (SOPs).

5 SUBJECT DISPOSITION

The number of subjects who are treated, complete the study, and reasons for discontinuation from the study will be summarized in tabular format for the safety population separately by subjects enrolled prior to 01Jan2022 and subjects enrolled after 01Jan2022. Subject disposition will also be displayed for the safety population in a subject listing.

6 DEMOGRAPHIC CHARACTERISTICS

Demographic and other characteristics will be summarized by treatment group and consist of (but will not be limited to) the following: age, sex, ethnicity, race, height, weight, and BMI.

For quantitative variables, summary statistics (n, mean, SD, minimum, first quartile, median, third quartile, and maximum) will be presented for the safety population. For qualitative variables, results will be summarized as counts and percentages for the stratified safety population. Demographic and other characteristics will be tabulated also for the mITT population.

Individual demographic and other characteristics for the safety population will be displayed in subject listings. A variable indicating enrollment stratification will be included in the listing.

7 MEDICAL HISTORY

All medical history events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) coding system (Version 25.0). Medical history events (past and current medical disorders) will be presented by treatment group for the safety population, summarized by MedDRA System Organ Class (SOC) and Preferred Term.

Medical history events will be displayed for the safety population in a subject listing. Specific medical history will also be summarized and listed for the safety population.

8 SURGICAL PLANS AND ASSESSMENTS

A frequency table will be presented by treatment group for first and second surgical approach and the presence (absence) of a stoma. The table will be sub-divided by subjects enrolled prior to 01Jan2022 and subjects enrolled on or after 01Jan2022. All surgery information will be listed for the safety population.

9 PRIOR AND CONCOMITANT MEDICATIONS

All medications will be coded using the World Health Organization Drug Dictionary (WHO Drug) version March 2022. Frequency tables will be presented by treatment group for prior and concomitant medications taken during the study.

- Prior medications are defined as those taken with a start date prior to the first split dose of study medication and ongoing is No and a stop date prior to the date of the first split dose of study medication.
    - Medications with an unknown stop date will be considered concomitant
- Concomitant medications are defined as those with a start date on or after the first dose of study medication, or those with a start date before the first dose of study medication and a stop date on or after the first dose of study medication (including those medications which are classified as ongoing).

All prior and concomitant medications will be listed for the safety population. All concomitant treatments or procedures will be listed for the safety population.

10 DRUG EXPOSURE

The number and percent of subjects who consumed all of one or both doses will be presented by treatment group for the safety population. The estimated volume of each dose received will be summarized by treatment group. Subject satisfaction with the taste will also be presented by treatment group for the safety population. Additionally, subject satisfaction will be further classified by those who completed both doses and those who did not within treatment group. All findings will be displayed in subject listings. A symmetric scatter plot matrix will be used to display via histograms and scatter plots the relationship between Time to GI2, Change in Adhesion Score, and Time from last dose to start of surgery. There will be one cell in a plot matrix for each stratum surgery type (laparoscopic, minimally invasive) and for each of the test articles (LB1148, placebo).

11 EFFICACY ANALYSIS

11.1 Primary Endpoints

11.1.1 Intra-Abdominal Adhesions

The primary efficacy endpoint for this study involves intra-abdominal adhesions and is as follows:

- Extent and severity of intra-abdominal adhesions as determined by the surgeon using the Intra-Abdominal Adhesion Extent and Severity Assessment Worksheet (Appendix 5 of the protocol)

If successful, this proof-of-concept study should provide necessary statistical information that when combined with information on clinical significance will allow for the computation of meaningful effect size and power calculations to support a subsequent phase 3 study(ies) with the following hypotheses: The null hypothesis for these primary efficacy endpoints is that there is no significant difference in intra-abdominal adhesions (extent and severity) between treatment groups; the alternative hypothesis is that there is a significant reduction in intra-abdominal adhesions (extent and severity) between treatment group~~s~~.

The presence or absence of adhesions, surgeon-recorded values for the extent and severity of visible intra-abdominal adhesions using the Intra-Abdominal Adhesion Extent and Severity (Appendix 5 of the protocol) will be recorded at both the first and second surgeries. The following nine variables will be calculated at both the first and second surgeries and summarized:

1. Summed Bowel-to-Abdominal Wall Adhesions Extent Score = bowel-to-abdominal wall extent scores summed across all 9 quadrants
2. Summed Bowel-to-Abdominal Wall Adhesions Severity Score = bowel-to-abdominal wall severity scores summed across all 9 quadrants
3. Bowel-to-Abdominal Wall Summed Extent and Severity Scores = add the Bowel-to-Abdominal Wall extent and severity scores together
4. Summed Bowel-to-Bowel Adhesions Extent Score = bowel-to-bowel extent scores summed across all 9 quadrants
5. Summed Bowel-to-Bowel Adhesions Severity Score = bowel-to-bowel severity scores summed across all 9 quadrants
6. Bowel-to-Bowel Summed Extent and Severity Scores = add the Bowel-to-Bowel extent and severity scores together
7. Bowel-to-Abdominal Wall and Bowel-to-Bowel Summed Extent and Severity Scores = add the Bowel-to-Abdominal Wall Summed Extent and Severity Scores (i.e., [3] above) to the Bowel-to-Bowel Summed Extent and Severity Scores (i.e., [6] above)
8. Take the Maximum of the Bowel-to-Bowel Severity Scores over all 9 regions
9. Bowel to Abdominal Wall Summed Severity Score added to the maximum Bowel to Bowel Severity Scores over the 9 regions, i.e., [2] and [8] are summed to obtain the Cocclini Peritoneal Adhesion Index (PAI)

Descriptive statistics as detailed in Section 4.2 as well as 95% confidence intervals will be provided for the nine extent and severity score detailed above across treatment groups and stratified by surgery type.

The summed extent and severity scores specified above (only # 7) will be compared between treatment groups at both the first and second surgeries, using a van Elteren test which allows for

the incorporation of the stratification factor type of surgery (laparotomy versus minimally invasive approaches) into the analysis. Additionally, the change from baseline in the summed extent and severity scores (i.e., from the first surgery to the second surgery) will be compared between treatment groups using a van Elteren test, again allowing control for surgery type. (This comparison will also be performed for that subgroup of subjects who had adhesions observed at the time of the first surgery.)

As stated in Section 4.2, all p-values obtained will be considered nominal.

All results will be summarized by treatment group for the mITT and PP populations. All findings will be presented in subject listings for the mITT population.

11.2 Secondary Endpoints

11.2.1 Intra-Abdominal Adhesions

The secondary efficacy endpoints for this study involve intra-abdominal adhesions and are as follows:

- Incidence of intra-abdominal adhesions at the second surgery

  Intra-abdominal lesions (Y/N) will be compared between treatment groups (LB1148 or Placebo) at both the first and second surgeries, analyzed using counts and percentages with comparisons made using the Cochran-Mantel-Haenszel (CMH) to obtain 95% Wald Confidence limits for the relative risk of adhesions along with the chi-square test, which allows for the incorporation of the stratification factor type of surgery (laparotomy versus minimally invasive approaches) into the analysis chi-square test for homogeneity of proportions. The change in proportion between the two surgeries will be evaluated across treatment and stratified by surgery type.
- Extent and severity of adhesions at the second surgery (Incorporated into the tables leading to the primary endpoint)
- Change from baseline in the incidence of intra-abdominal adhesions (Simple shift table)
- Compare change from baseline in incidence, extent, and severity of intra-abdominal adhesions among subjects treated with LB1148 or placebo who had adhesions observed at the time of the first surgery. (A subset of the primary endpoint analysis.)
- Compare number of adhesions between the LB1148 and placebo groups
- Compare change between the two surgeries in the number of adhesions between the LB1148 and placebo groups

11.2.2 Bowel Function Endpoints

The secondary efficacy endpoints include the number of hours from surgical closure to resolution or presence, as appropriate, of:

- Achievement of GI2, defined as the toleration of solid food (subject finished a meal that required chewing and experienced no significant nausea/vomiting for four hours) and the

recovery of the upper and lower GI tract (first bowel movement; passage of stool) following surgery (the time the last skin staple or suture was placed by the surgeon)
- Nausea with a Verbal Rating Score (VRS) ≥3 (moderate to severe) (Listing only)
- Vomiting or retching* (Listing only)
- Presence of flatus (Listing only)
- Bowel movement (presence of stool from anus or ostomy)
- Insertion and reinsertion of nasogastric (NG)/orogastric (OG) tube*
    - Removal of NG/OG tube* (Listing only)
- Ability to tolerate a liquid oral diet (liquid diet ordered, and subject is able to sustain oral liquid intake without need for IV fluid administration for hydration)
- Ability to tolerate a solid oral diet (solid food ingestion without significant nausea/vomiting for four hours)
- Achievement of GI3, identical to GI2 except recovery of the upper and lower GI tract is defined by either bowel movement or flatus
- Resolution of POI, defined as having resolved when all of the following criteria are met:
    - Absence of nausea AND 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

The following POI parameters will also be evaluated:

- Incidence of POI (Within 14 days of first surgery, After Discharge), defined as the greater than expected inability to tolerate liquids or solids during the post-operative period
    - Within 14 days of first surgery subjects will be identified by AE preferred term (PT) **and** by a confirmatory imaging study within the start/stop dates of the POI
        - The AE PTs are ileus, post-operative ileus, small bowel obstruction, bowel obstruction, partial bowel obstruction, mechanical bowel obstruction, and non-mechanical bowel obstruction
- Incidence of POI after hospital discharge
    - Subjects will be identified by AE PT as listed above

*Vomiting and retching, as well as insertion, reinsertion, and removal of NG/OG tube will be treated as binary (yes/no) variables.

Selected time-to-event secondary efficacy endpoints (See Table in Section 11.5) will be analyzed using a stratified Cox PH model with treatment as the main effect, stratified by surgery type (laparotomy versus minimally invasive). In addition to surgery type, a categorical age (<65 years, >= 65 years) will be included. Nominal p-values will be reported. Comparison of treatment groups and assessment of the significance of the parameter estimates for surgery type will be made using the Wald chi-square test.

In addition to the semi-parametric Cox PH model, non-parametric Kaplan-Meier survival curves will be produced for the two treatment groups and compared using a stratified log-rank test; results from this test will be considered nominal/hypothesis-generating in nature. The number of hours to each endpoint will be presented in summary tables with descriptive statistics for the mITT (all variables selected) and PP populations (POI, GI2, and GI3 variables only).

Those endpoints defined as yes/no variables will be analyzed using a chi-square test for homogeneity of proportions.

All findings will be presented in subject listings for the mITT population, including calculated time to event efficacy variables.

11.3 Exploratory Endpoints

The exploratory endpoints are:

- Hospital LOS (number of days in the hospital, evaluated in hours) will be measured first by time to discharge order is written. The time will start at time of surgical closure and run until discharge order is written.

- Pain values, evaluated using
    - Values recorded from the NPRS
    - Analgesia consumption, including totals for opioid use and morphine equivalents
        - See Section 15 APPENDIX A for conversion guidelines
- The number of subjects with recorded bowel obstructions (Listing only)
    - AE Preferred Terms having the phrase ”bowel obstruction” in them e.g., small bowel obstruction
- The number of subjects with a hospital re-admission
- Blood product transfusion volume on the day of surgery (Listing only)

The following LOS parameters will also be evaluated:

- Time from surgical closure to actual discharge
- Time from surgical closure to standard discharge (Listing only) The time will start at time of surgical closure and run until all four criteria for discharge have been met. The criteria are:
    - Passage of stool
    - Ability to tolerate solid food and drink comfortably
    - Adequate oral analgesia, and
    - Subject’s willingness to be discharged

Selected time-to-event endpoints (See Table in Section11.5) will be analyzed using a stratified Cox PH model with treatment as the main effect, stratified by surgery type (laparotomy versus minimally invasive). Comparison of treatment groups and assessment of the significance of the

parameter estimates for surgery type will be made using the Wald chi-square test. In addition to the semi-parametric Cox PH model, non-parametric Kaplan-Meier survival curves will be produced for the two treatment groups and compared using a stratified log-rank test. The number of hours to each endpoint will be presented in summary tables with descriptive statistics for the mITT and PP populations.

Pain assessments will be collected daily from surgery (Day 0) through post-operative Day 14 (or for as long as the patient remains hospitalized), at hospital discharge, and at the second surgery.

All exploratory endpoints will be summarized by treatment group, except where it is indicated that only a listing will be provided. For continuous endpoints, summary statistics (n, mean, SD, minimum, first quartile, median, third quartile, maximum) will be presented for the mITT population, with comparisons of means made using the van Elteren test (Justification previously provided in Section 11.1.1.) For categorical endpoints, counts and percentages will be presented for the mITT population, with comparisons of proportions made using CMH with Wald 90% CIs (Justification previously provided in Section 11.1.111.1.1).

All results will be listed for the mITT population, including calculated time to event efficacy variables.

11.4 Censoring Rules

Condition (within 14 days of surgery)	**Censoring**
No(t) Z[1] but death report	Censoring at Study Day 14
No(t) Z[1] and the subject is lost to follow-up or withdrew informed consent	Censoring at Study Day 14
No(t) Z[1] within 14 days after surgery	Censoring at Study Day 14

[1]Where Z is GI2, GI3, bowel movement, liquid oral diet, solid oral diet, discharge order written, actually discharged from hospital

11.5 Efficacy Time-to-Event Parameter by Analysis Type

Number of hours to Analysis	**Cox PH**		**Kaplan-Meier**		**Other**	
	mITT	PP	mITT	PP	mITT	PP
GI2	Yes	Yes	Yes	Yes	Frequency Table	Frequency Table
Bowel movement within 14 days	Yes	No	Yes	No	Frequency Table	No
NG/OG use (insertion, reinsertion)	No	No	No	No	Frequency Table only	No
NG/OG removal	No	No	No	No	Listing only	No
Liquid oral diet	No	No	Yes	No	Frequency Table	No
Solid oral diet	Yes	No	Yes	No	Frequency Table	No
GI3	Yes	Yes	Yes	Yes	Frequency Table	Frequency Table
POI within 14 days	Yes	Yes	Yes	Yes	Frequency Table	Frequency Table

Number of hours to Analysis	Cox PH		Kaplan-Meier		Other	
POI after discharge	No	No	No	No	Included in Frequency Table for POI within 14 days	No
Discharge order written	Yes	Yes	Yes	Yes	No separate descriptive Frequency Table	No separate descriptive Frequency Table
Actual discharge	Yes	Yes	Yes	Yes	No separate descriptive Frequency Table	No separate descriptive Frequency Table
Standard discharge	No	No	No	No	Listing only	No
Nausea	No	No	No	No	Listing only	No
Vomiting	No	No	No	No	Listing only	No
Flatus	No	No	No	No	Listing only	No

12 SAFETY ANALYSIS

12.1 Adverse Events

All adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) coding system (Version 25.0). Frequency tables will be presented by treatment group for all adverse events (AEs) by system organ class and preferred term for the safety population. Frequency tables will also be presented for AEs leading to discontinuation of test article, AEs by maximum severity (per CTCAE Version 5.0 Section 7.11 Study Protocol), AEs related to test article, and serious AEs. Subject listings of all AEs will also be provided for the safety population.

In all displays, adverse events will be displayed by MedDRA System Organ Class (SOC) and Preferred Terms. Subjects who have the same AE occur more than once will be counted only once for that event. Subjects who have more than one AE within a system organ class will be counted only once in that system organ class. (The addition of severity or relatedness follow the same pattern.)

In addition, frequency tables by treatment group for all AEs by system organ class and preferred term will be presented for the following populations:

- Patients who have had chemotherapy in the last 60 days
- Patients with a venous port or catheter
- Patients with varicose veins and superficial vein thrombosis
- Patients with varying degrees of obesity
    - Baseline BMI 30-35
    - Baseline BMI 35-40
- Patients who are smokers
- Patients with varying degrees of renal function

  - Baseline eGFR 45-59
  - Baseline eGFR 30-44
  - Baseline eGFR 15-29
  - Baseline eGFR < 15
- Patient age groups
  - 18-64 years
  - 65-80 years

12.2 Laboratory Tests

Safety labs are performed at Screening and on post-operative Day 3, and post-operative Day 7 (if subject is still hospitalized). For hematology, chemistry, and coagulation, summary statistics (n, mean, SD, minimum, median, and maximum) will be presented for the observed values at each time point for the safety population. Summary statistics will also be presented for the change from baseline values to each post-baseline time point. In these displays, baseline will be defined as the last values obtained prior to the first dose.

Pregnancy tests are performed at Screening and before each surgery.

For all tests, including pregnancy tests, results will be displayed in subject listings for the safety population. Laboratory reference ranges will be provided by the laboratory site and will be included in an appendix of the clinical study report.

12.3 Physical Examinations

Complete physical examinations are performed from Screening through post-operative Day 14 (or for as long as the patient remains hospitalized), at hospital discharge, and at the second surgery (if applicable). Each physical examination includes weight measurements and lower extremity assessments to identify any clinical symptoms of venous thromboembolism (VTE). Height is measured at the Screening visit only.

A frequency table of lower extremity assessment findings will be presented by treatment group for the safety population.

All physical exam and lower extremity assessment findings will be displayed in subject listings for the safety population.

12.4 Vital Signs

Vital signs will be collected daily from Screening through post-operative Day 14 (or for as long as the patient remains hospitalized), at hospital discharge, and at the second surgery. Summary statistics (n, mean, SD, minimum, median, and maximum) will be presented for the observed values at each time point for systolic and diastolic blood pressure, heart rate, respiratory rate, oxygen saturation, and temperature at each time point for the safety population. Summary statistics will also be presented for the change from baseline values to each post-baseline time point. In these displays, baseline will be defined as the last value obtained prior to the first dose.

All vital signs results will be displayed in subject listings for the safety population.

13 SUBJECT LISTINGS

All data that are collected and entered into the study database will be presented in subject listings.

14 INTERIM ANALYSES

An interim analysis of limited efficacy, safety, and tolerability was proposed in the initial study protocol. The purpose of the interim analysis was to allow for sample size adjustment based on observed data, e.g., an increase in sample size, if needed. An interim analysis with primary endpoints return of bowel function (GI2) and LOS (Discharge Order Written) was performed in the spring of 2020. Efficacy and safety tables were provided to the sponsor in May of 2020.

The change in primary endpoint with Protocol Amendment #5 modified the emphasis of this study. No change in sample size was made because the endpoints reviewed were no longer the primary endpoints. As previously discussed in Section 4.1, the subjects used for this analysis are now included only in the safety population.

15 APPENDIX A

MME Calculator Guidance

1. Determine the total daily amount of each opioid the subject takes
2. Convert each to MMEs by multiplying the dose for each opioid by the conversion factor (see table below)
3. Add them together

Use the formula: Strength per unit (*units in table below*) X (number of units/day) X MME factor = MME/day

Example for Hydrocodone 5/325, 2 units BID: 5mg X 4 X 1 = 20 MME/day

Type of Opioid (strength units)	**MME Conversion Factor**
Buprenorphine film/tablet[iii] (mg)	
Buprenorphine[iii] (mcg/hr)	
Buprenorphine[iii] (mcg)	
Butorphanol (mg)	7
Codeine (mg)	0.15
Dihydrocodeine (mg)	0.25
Fentanyl buccal or SL tablets, or lozenge/troche[iv] (mcg)	0.13
Fentanyl film or oral spray[v] (mcg)	0.18
Fentanyl nasal spray[vi] (mcg)	0.16
Fentanyl patch[vii] (mcg)	7.2
Hydrocodone (mg)	1
Hydromorphone (mg)	4
Levorphanol tartrate (mg)	11
Meperidine hydrochloride (mg)	0.1
Methadone[viii] (mg)	3
>0, ≤20	4
>20, ≤40	8
>40, ≤60	10
>60	12
Morphine (mg)	1
Opium (mg)	1
Oxycondone (mg)	1.5
Oxymorphone (mg)	3
Pentazocine (mg)	0.37
Tapentadol[ix] (mg)	0.4
Tramadol (mg)	0.1

i The MME conversion factor is intended only for analytic purposes where prescription data are used to calculate daily MME. Use the formula: Strength per Unit X (Number of Units/ Days

Supply) X MME conversion factor = MME/Day. This value does not constitute clinical guidance or recommendations for converting patients from one form of opioid analgesic to another. Please consult the manufacturer's full prescribing information for such guidance. Use of this file for the purposes of any clinical decision-making warrants caution. This is particularly true with regard to methadone (see viii below).

ii National Center for Injury Prevention and Control. CDC compilation of benzodiazepines, muscle relaxants, stimulants, zolpidem, and opioid analgesics with oral morphine milligram equivalent conversion factors, 2017 version. Atlanta, GA: Centers for Disease Control and Prevention; Available at https://www.cdc.gov/drugoverdose/resources/data.html . For more information, send an email to Mbohm@cdc.gov" Mbohm@cdc.gov.

iii Buprenorphine products are listed but do not have an associated MME conversion factor. These buprenorphine products, as partial opioid agonists, are not expected to be associated with overdose risk in the same dose-dependent manner as doses for full agonist opioids. The conversion factors for drugs prescribed or provided as part of medication-assisted treatment for opioid use disorder should not be used to benchmark against dosage thresholds meant for opioids prescribed for pain.

iv The MME conversion factor for fentanyl buccal tablets, sublingual tablets, and lozenges/troche is 0.13. This conversion factor should be multiplied by the number of micrograms in a given tablet or lozenge/troche.

v The MME conversion factor for fentanyl film and oral spray is 0.18. This reflects a 40% greater bioavailability for films compared to lozenges/tablets and 38% greater bioavailability for oral sprays compared to lozenges/tablets.

vi The MME conversion factor for fentanyl nasal spray is 0.16, which reflects a 20% greater bioavailability for sprays compared to lozenges/tablets.

vii The MME conversion factor for fentanyl patches is based on the assumption that one milligram of parenteral fentanyl is equivalent to 100 milligrams of oral morphine and that one patch delivers the dispensed micrograms per hour over a 24 hour day. Example: 25 ug/hr fentanyl patch X 24 hrs = 600 ug/day fentanyl = 60 mg/day oral morphine milligram equivalent. In other words, the conversion factor not accounting for days of use would be 60/25 or 2.4. However, since the fentanyl patch remains in place for 3 days, we have multiplied the conversion factor by 3 (2.4 X 3 = 7.2). In this example, MME/day for ten 25 µg/hr fentanyl patches dispensed for use over 30 days would work out as follows: Example: 25 ug/hr fentanyl patch X (10 patches/30 days) X 7.2 = 60 MME/day. Please note that because this allowance has been made based on the typical dosage of one fentanyl patch per 3 days, you should first change all Days Supply in your prescription data to follow this standard, i.e., Days Supply for fentanyl patches= # of patches X 3.

viii The CDC MME conversion factor to calculate morphine milligram equivalents of methadone is 3. Calculating MME for methadone in clinical practice often involves a sliding-scale approach whereby the conversion factor increases with increasing dose since the conversion factor of 3 for methadone could underestimate MME for a given patient. CMS uses this conversion factor when analyzing Medicare population opioid use. CMS uses the graduated methadone MME conversion factors to calculate MME within the Overutilization Monitoring System (OMS) for identifying

and reporting potential opioid overutilizers.
https://www.cdc.gov/drugoverdose/pdf/calculating_total_daily_dose-a.pdf.

ix Tapentadol is a mu receptor agonist and norepinephrine reuptake inhibitor. Oral MMEs are based on degree of mu receptor agonist activity, but it is unknown if this drug is associated with overdose in the same dose-dependent manner as observed with medications that are solely mu receptor agonists.

16 FINAL SIGN-OFF FOR PALISADE BIO LBS-POI-201 STATISTICAL ANALYSIS PLAN

[redacted]
Biostatistician
Innovative Analytics

Date

[redacted]
VP Clinical Operations
Palisade Bio

Date

17 REVISIONS TO STATISTICAL ANALYSIS PLAN

Date	Revision
October 11, 2022	Version 2.0: Extensive revisions reflect the changes made to the study protocol covering Amendments 4, 5, and 6. The most significant change was the in the designation of the primary endpoint. The primary endpoint was changed from the number of hours from surgical closure to return of GI function as measured by GI2 to compare the change from baseline in extent and severity of intra-abdominal adhesions among subjects treated with LB1148 or placebo. Another significant change increased the alpha to 0.10 and changed the CIs from 95% to 90%.
March 10, 2023	Version 3.0: The following statement was returned to this version of the SAP. "All statistical tests will be 2-sided, with p-values considered nominal. A p-value < 0.05 will be highlighted." Gender and BMI added to models in specific efficacy analyses. An analysis was added regarding time between drug administration and actual surgery start time and efficacy between the two treatment groups. Confidence intervals were changed from 90% back to 95% throughout. Cocclini scoring system added to the primary endpoint. One shift table was added to better display the change in number of adhesions between the two surgeries.