STATISTICAL ANALYSIS PLAN (SAP)

Protocol Number:	PBI-POI-301
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)
Product Name or Number:	LB1148
Sponsor:	Palisade Bio, Inc. 5800 Armada Drive, Suite 210 Carlsbad, CA 92008
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SAP Version Number (Date):	Version 0.2 (July 14, 2022)
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1 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Term	Definition
AE	adverse event
CMH	Cochran-Mantel-Haenszel
DOW	discharge order written
GI	gastrointestinal
IcE	intercurrent events
ICH	International Council for Harmonization
IP	investigational product
ITT	intent-to-treat
LB1148	investigational product - the active ingredient, TXA, is formulated in a solution containing PEG, glucose, electrolytes, and water
LOS	length of stay
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent-to-treat
MME	morphine milligram equivalent
PH	proportional hazards
POI	post-operative ileus
PP	per protocol
PT	preferred term
SAE	serious adverse event
SD	standard deviation
SEM	standard error of the mean
SOC	system organ class
SOP	standard operating procedure
TAD	time the subject is actually discharged from the hospital
TDO	time that the hospital discharge order is written
Y/N	Yes/No

2 STUDY OVERVIEW

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 to surgery. Subjects will then undergo surgery (Study 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 post-operative ileus (POI) through Study Day 90.

The study will evaluate return of gastrointestinal (GI) function, as provided in the primary objective (Section 3.1), in order to determine the overall study drug effect. Additionally, an evaluation of the secondary (Section 3.2) and exploratory objectives (Section 3.3) related to return of GI function, length of stay (LOS), POI, and opiate use will be performed.

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

This study is to be conducted at up to approximately 30-60 sites in the United States, with planned enrollment of 650 subjects. Unless required for safety reasons or protocol compliance, Sponsor personnel, all subjects, Investigators, Pharmacists, and persons performing assessments, will remain blinded to the identity of the treatment from time of randomization until final database lock.

An independent data monitoring committee (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.

3 STUDY OBJECTIVES

3.1 Primary Objective

The primary objective is to compare the 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 last, among subjects treated with LB1148 or placebo.

3.2 Secondary Objectives

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

- Time to first bowel movement
- LOS Ready, 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 Discharge Order Written (DOW) defined as the time from the end of surgery to the time that the hospital discharge order is written (TDO)
- LOS Actual - defined as the time from the end of surgery to the time the subject is actually discharged from the hospital (TAD)

3.3 Exploratory Objectives

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

- Time to GI-3, defined as the time from the end of surgery to the time as the of toleration of solid food and either first flatus or bowel movement, whichever occurs last
- Incidence of the following events at Day 90:
    - Hospital readmission
    - POI
- Opiate use by morphine milligram equivalent (MME)

3.4 Safety Objectives

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

4 GENERAL METHODS

4.1 Analysis Populations

Intent-to-Treat Population (ITT): The ITT population includes all randomized subjects.

modified Intent-to-Treat Population (mITT): The mITT 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, The surgery must occur for

the efficacy endpoints to be measured and meaningful. All analyses corresponding to the primary endpoint and all secondary endpoints will be formally assessed using the mITT population.

Safety Population: Ideally, the safety population will be defined as all randomized subjects who receive any amount of IP. If a subject were to receive IP without being randomized, then they would be included in the Safety population also. Subjects will be analyzed according to the treatment actually received.

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

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 (or geometric mean), standard deviation (SD), standard error of the mean (SEM), 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 ITT (all efficacy endpoints) and PP populations (primary and secondary efficacy endpoints), while the safety analyses will be performed for the safety population. All statistical tests will be 2-sided with an alpha of 0.05.

4.3 Sample Size Justification and Randomization

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.

The table below displays the results of sample size calculations for the differences between treatment groups in median times to return of bowel function ranging from 25 to 18 hours; all for a 2-sided 0.05 significance level and the log-rank test:

Assumed MTTRBF (hrs)				**Required n per group**	
Placebo	**LB1148**	**Diff (hrs)**	**HR**	**80% power**	**90% power**
92	67	25	1.373	167	223
92	68	24	1.353	183	245
92	69	23	1.333	202	271
92	70	22	1.314	224	300
92	71	21	1.296	250	334
92	72	20	1.278	279	373
92	73	19	1.260	313	419

92	74	18	1.243	354	474

MTTRBF = median time to return of bowel function
HR = hazard ratio = MTTRBF (Placebo) / MTTRBF (LB1148) (assuming exponential survival curves for both treatment groups)

For randomization purposes, subjects will be stratified by 1) surgery type (laparotomy versus minimally invasive approaches) and 2) planned stoma (Yes/No) (Y/N)).

4.4 Data Handling

4.4.1 Method for Handling Missing Data

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.

4.4.2 Definition of Baseline Values

Unless otherwise specified, Baseline values for vital signs, physical examinations, and concomitant medications are defined as the last values collected before the first dose of IP. Unless specified otherwise, the efficacy endpoints begin at the end of surgery.

4.4.3 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).

4.4.4 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 will be considered using the Hochberg procedure. The p-values for each secondary endpoint will be computed and then ordered from largest to smallest (Hochberg 1988). These ordered p-values will then be compared to the α critical values 0.05, 0.025, 0.0167, and 0.0125, respectively. If the first p-value 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.

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 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.

6 DEMOGRAPHIC CHARACTERISTICS

Demographics, baseline, and surgical characteristics will be summarized by treatment group and consist of (but are 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, baseline, and surgical characteristics will be summarized for both the safety population and ITT population.

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 (PT), and results presented by treatment group for the safety population. All medical history events will be displayed for the safety population in a subject listing.

8 SURGICAL PLANS AND ASSESSMENTS

Frequency tables will be presented by treatment group for initial surgical plan information and surgery assessments. 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 (WHODrug) version currently in use at the start of the study. 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 dose of study medication.
- 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.

10 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. All findings will be displayed in subject listings.

11 EFFICACY ANALYSIS

11.1 Primary Efficacy Endpoint

11.1.1 Time to GI-2

The primary endpoint is the 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 a subject finished a meal that required chewing and experienced no significant nausea/vomiting for 4 hours after the solid meal.

11.1.1.1

  - 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.

Analysis Methods

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 proportional hazards (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 leu 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 (IcE)

11.1.2 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 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 1, along with the related censoring rules for each analysis.

Table 1 IcE for Time to GI-2

	Primary Analysis Approach (Kaplan-Meier[1] and Cox Models[2])		**Supplemental Analyses (Kaplan-Meier[1] Model Only)**	
Intercurrent Event	**First Run of Model**	**Second Run of Model**	**First Run of Model**	**Second Run of Model**
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)

	Primary Analysis Approach (Kaplan-Meier[1] and Cox Models[2])		Supplemental Analyses (Kaplan-Meier[1] Model Only)	
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

[1]Kaplan-Meier with log rank test

[2]Cox PH model with Wald chi square

*Descending Event Dates: All missing values for subjects on placebo due to IcE 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.

Descending event dates for the controls with missing intercurrent event data will document what would happen if the missing data in the treatment group subjects had worse outcomes to that of the missing data in the control group. The treatment group data is censored at Day 16 while the censoring dates for the control group subjects are systematically decreased (Study Day 15, Study Day 14, and Study Day 12).

11.1.1.3 **Tabulation of Patterns of Missing Data**

To visualize any patterns present in the missing data, the following table will be constructed: The number of subjects with no GI-2 data due to death, lost to follow-up or withdrew, or not achieved within 14 days will be cross tabulated by treatment (LB1148 or placebo) within the four cells making up the randomization strata, e.g., type of surgery (laparotomy versus minimally invasive approaches) crossed with stoma construction (Y, N).

11.1.1.4 **Partial Dates and Times**

All components of the primary endpoint, time to GI-2, are collected while the subject is in the hospital, so the dates should be available. See Section 4.4.1 for times which may be imputed.

11.2 Secondary Efficacy Endpoints

11.2.1 Time to First Bowel Movement

The secondary endpoint, time to first bowel movement, is defined as the time in hours from placement of the last skin staple or suture to the time of first bowel movement.

Analysis Methods

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.

11.2.1.1 **Estimand and Associated IcE**

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 analysis (which includes a 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.

11.2.1.2

The IcEs that may affect the interpretation or the occurrence of this estimand are tabulated below in Table 2, along with the related censoring rules for each analysis.

Table 2 IcE for Time to First Bowel Movement

Intercurrent Event	Primary Analysis Approach (Kaplan-Meier[1] and Cox Models[2])		Supplemental Analysis (Kaplan-Meier[1]) Model Only)
	First Run of Cox Model	Second Run of Cox Model	
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 withdrew informed consent	Censoring at Study Day 16	Censoring at Study Day 16	Censoring at the date consent withdrawn
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

11.2.1.3

[1]Kaplan-Meier with log rank test
[2]Cox PH model with Wald chi square
*The second Cox PH model adding "Any opiates" use will be performed only if the strata are significant for the primary endpoint analyses

Tabulation of Patterns of Missing Data

To visualize any patterns, present in the missing data the following table will be constructed: The number of subjects with no bowel movement due to death, lost to follow-up or withdrew, or not achieved within 14 days will be cross tabulated by treatment (LB1148 or placebo) within the

four cells making up the randomization strata, e.g., type of surgery (laparotomy versus minimally invasive approaches) crossed with stoma construction (Y/N).

Partial Dates and Times

All components of the secondary endpoint, time to first bowel movement, are collected while the subject is in the hospital, so the dates should be available. See Section 4.4.1 for times which may be imputed.

11.2.1.4

11.2.2 LOS – Ready, DOW, and Actual

Analysis Methods

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
11.2.2.1 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 IcE

11.2.2.2 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 3, along with the related censoring rules for each analysis.

Table 3 IcE for LOS

Intercurrent Event	Primary Analysis Approach (Kaplan-Meier[1] and Cox Models[2])		Supplemental Analyses (Kaplan-Meier[1]` Model Only)
	First Run of Cox Model	Second Run of Cox Model*	
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

Intercurrent Event	Primary Analysis Approach (Kaplan-Meier[1] and Cox Models[2])		Supplemental Analyses (Kaplan-Meier[1]` Model Only)
	First Run of Cox Model	Second Run of Cox Model*	
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

[1]Kaplan-Meier with log rank test

[2]Cox PH model with Wald chi square

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

11.2.2.3

Tabulation of Patterns of Missing Data

To visualize any patterns, present in the missing data the following table will be constructed: The number of subjects with failure to meet discharge criteria (Ready, DOW, Actual) data due to death, lost to follow-up or withdrew, or not achieved within 14 days will be cross tabulated by treatment (LB1148 or placebo) within the four cells making up the randomization strata, e.g.,
11.2.2.4 type of surgery (laparotomy versus minimally invasive approaches) crossed with stoma construction (Y, N).

Partial Dates and Times

All components of the secondary endpoint(s), failure to meet discharge criteria (Ready, DOW, Actual), are collected while the subject is in the hospital, so the dates should be available. See Section 4.4.1 for times which may be imputed.

11.3 Exploratory Efficacy 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 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). Time to GI-3 will be analyzed using time to event methods described above for the primary endpoint, time to GI-2. If a date is present but a time is absent, time may be imputed as described in Section 4.4.1.

- Proportion of subjects not readmitted to the hospital following initial discharge by Study Day 90 will be analyzed using counts and percentages with comparisons made using the Cochran-Mantel-Haenszel (CMH) chi-square test, which allows for the inclusion of the stratification factors type of surgery (laparotomy versus minimally invasive approaches) crossed with stoma construction (Y, N).
- Proportion of subjects without POI will be analyzed using counts and percentages with comparisons made using the CMH chi-square test, which allows for the inclusion of the stratification factors type of surgery (laparotomy versus minimally invasive approaches) crossed with stoma construction (Y, N). The following definitions of POI will be used:
  - 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 is 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.
- Total opiate use by aggregate MME from the end of surgery until Actual discharge or Day 16, whichever is earlier. 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.

All results will be listed for the ITT population.

12 SAFETY ANALYSIS

12.1 Adverse Events

All AEs will be coded using the MedDRA coding system. Frequency tables will be presented by treatment group for all AEs by SOC and PT for the safety population. Frequency tables will also be presented for AEs leading to discontinuation of IP and study, AEs by maximum severity, AEs by causality, and serious AEs. Subject listings of all AEs will also be provided for the safety population.

In all displays, AEs will be displayed by MedDRA SOC and PT. 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 SOC will be counted only once in that SOC. No formal statistical testing will be done.

12.2 Laboratory Tests

Safety labs are performed at Screening and on post-operative Day 3. For hematology, chemistry, and coagulation, summary statistics (n, mean, SD, SEM, 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.

Laboratory abnormalities will be analyzed as safety outcomes by summarizing frequency, severity, and changes from baseline.

Pregnancy tests are performed at Screening and before 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 at Screening, and targeted physical exams are performed at Day 2, then daily through post-operative Day 16 (or for as long as the patient remains hospitalized), including at hospital discharge. Complete physical examinations include examination of the following systems: cardiovascular, dermatological, ear, nose, and throat, extremities, GI, musculoskeletal. ophthalmological, neurological, and respiratory. Targeted physical examinations are brief, focused examinations of the subject following medical history and include an assessment of lower extremities for possible venous thromboembolism and an assessment for AEs.

Height and weight are measured at the Screening visit only.

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

All findings will be displayed in subject listings for the safety population.

12.4 Vital Signs

Vital signs will be collected at Screening, Day 2, then daily through post-operative Day 16 (or for as long as the patient remains hospitalized), including at hospital discharge. Summary statistics (n, mean, SD, SEM, 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 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

No interim analysis is planned.

15 FINAL SIGN-OFF FOR PALISADE BIO, INC PROTOCOL PBI-POI-301 STATISTICAL ANALYSIS PLAN

[redacted]
Biostatistician
Innovative Analytics

Date

[redacted]
Chief Medical Officer
Palisade Bio, Inc.

Date

16 REVISIONS TO STATISTICAL ANALYSIS PLAN

Date	Revision	Statistician's Signature
•	•	•
•	•	•
•	•	•
•	•	•
•	•	•
•	•	•