POLYPID LTD

STATISTICAL ANALYSIS PLAN (SAP)

Phase III, Prospective, Multinational, Multicenter, Randomized, Controlled, Two-arm, Double Blind Study to Assess Efficacy and Safety of D-PLEX Administered Concomitantly with the Standard of Care (SoC), Compared to a SoC Treated Control Arm, in Prevention of Post Abdominal Surgery Incisional Infection

Study: D-PLEX 311

Phase: III

SAP Date: 16-JUN-2022

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Prepared by: [redacted]

Version Control Page

Version:	Version Date:	Comments
1	07-MAY-2020	Initial version
2	19-AUG-2020	Second version: to update following FDA recommendations and to align with changes in protocol version 04
3	28-APR-2022	To update following protocol amendment #6 and provide further clarifications and details as required.
4	16-JUN-2022	To document the adjusted significance level for the final analysis of the primary endpoint; to include additional subgroup analysis and additional exploratory analysis.

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GLOSSARY OF ABBREVIATIONS

AE	Adverse Event
ASEPSIS	Additional treatment, Serous discharge, Erythema, Purulent exudate, Separation of deep tissue, Isolation of bacteria, Stay duration as inpatient
ATC	Anatomical Therapeutic Chemical
AUC	Area Under the Curve
BMI	Body Mass Index
COPD	Chronic Obstructive Pulmonary Disease
CRF	Case Report Form
CS	Clinically Significant
CSR	Clinical Study Report
DMC	Data Monitoring Committee
DSSI	Deep Incisional Surgical Site Infection
FAS	Full Analysis Set
FDA	Food and Drug Administration
ICF	Informed Consent Form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IP	Investigational Product
ITT	Intention to Treat
IWRS	Interactive Web Randomization System
mITT	Modified Intention to Treat
MedDRA	Medical Dictionary for Regulatory Activities
OR	Operation Room
PE	Physical Examination
PI	Principal Investigator
PK	Pharmacokinetics
PLEX	Polymer-Lipid Encapsulation matrix
PP	Per Protocol
PT	Preferred Term
PVD	Peripheral Vascular Disease
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SE	Standard Error

SoC	Standard of Care
SOC	System Organ Class
SOP	Standard Operating Procedure
SSI	Surgical Site Infection
SSR	Sample Size Re-assessment
SSSI	Superficial Incisional Surgical Site Infection
TEAE	Treatment Emergent Adverse Event
WHO-DD	World Health Organization Drug Dictionary

1. PREFACE

This Statistical Analysis Plan (SAP) describes the planned analysis and reporting for PolyPid study D-PLEX 311, (phase III, prospective, multinational, multicenter, randomized, controlled, two-arm, double blind study to assess efficacy and safety of D-PLEX administered concomitantly with the Standard of Care (SoC), compared to a SoC treated control arm, in prevention of post abdominal surgery incisional infection), and was written in accordance with SOP 100-60-02 (Statistical Analysis Plan Preparation, Review and Approval, Bioforum's Procedure).

The structure and content of this SAP provides sufficient detail to meet the requirements identified by the FDA and International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH): E9 Guidance on Statistical Principles in Clinical Trials. All work planned and reported for this SAP will follow internationally accepted guidelines, published by the American Statistical Association, and the Royal Statistical Society, for statistical practice.

The following documents were reviewed in preparation of this SAP:

- Clinical Study Protocol D-PLEX 311 Version 06, issued on 22-FEB-2022
- Case Report Forms (CRFs) for Study D-PLEX 311
- ICH E9 Guidance on Statistical Principles for Clinical Trials
- ICH E3 Structure and Content of Clinical Study Reports
- Conduct of Clinical Trials of Medical Products During the COVID-19 Public Health Emergency, Guidance for Industry, Investigators, and Institutional Review Boards

The reader of this SAP is encouraged to also read the clinical protocol for details on the conduct of this study, and the operational aspects of clinical assessments and timing for completing a subject in this study.

A separate Interim Statistical Analysis Plan (ISAP) accompanies and completes this document with the full details about the stopping rules and the sample size decision rules for the planned interim analysis.

This SAP describes the statistical analyses as it is foreseen at the time of planning the study. The SAP serves as a compliment to the protocol and supersedes it in case of differences. In case of major differences between the protocol and SAP, a protocol amendment will be considered. Any deviations from the statistical analyses planned in the protocol will be documented in the SAP and any deviations from the statistical analyses planned in the SAP will be documented in the final clinical study report (CSR).

The SAP may be updated during the study conduct and will be finalized before breaking the blind of the study and before the database lock for interim analysis.

Version 2 is performed early during initiation of the study, responding to FDA recommendations and include additional clarifications and edits and alignment to protocol

version 04. Version 3 is performed in accordance with the issued protocol amendment version 06, while the study is ongoing, and the Sponsor is fully blinded.

2. STUDY OBJECTIVES

The primary objectives of this study are as follows:

- To assess the anti-infective efficacy of D-PLEX, administered concomitantly with Standard of Care (SoC), over a period of 30 days post operation, by preventing surgical site infection (SSI), defined as superficial and/or deep abdominal wall surgical incision infection, compared to the SoC treated control arm.
- To assess the safety of D-PLEX.

3. STUDY DESIGN

3.1. General Design and Study Schema

This is a phase III, prospective, multinational, multicenter, randomized, controlled, two-arm, double blind study. The study population includes male and female subjects, 18 years old and above at screening, undergoing an elective colorectal surgery involving resection, with or without a stoma formation, that includes at least 1 abdominal incision that is > 10cm (target incision).

Subjects who meet the inclusion criteria and none of the exclusion criteria and who provide a signed Informed Consent Form will be enrolled in the study.

Subjects will be randomized into either the investigational arm (D-PLEX + SoC) or to the control arm (SoC only) in a 1:1 ratio. Subjects will be stratified by prophylactic Standard of Care (prophylactic IV antibiotic with mechanical bowel preparation or prophylactic IV antibiotic without mechanical bowel preparation) and by region (USA or Europe+Israel).

The sponsor, the outcome assessor, the subjects and all staff involved in the collection and recording of the clinical and laboratory data, based on which the independent adjudication committee will perform their assessment, will be blinded to the treatment assignment. The operation room (OR) staff will be trained to maintain in confident the treatment assignment and not to disclose it to other staff. In addition, all aspects of data management and clean-up will be done using blinded datasets.

For subjects randomized to the investigational arm, D-PLEX treatment will be applied during the abdominal surgery at the stage of closure of the abdominal wall surgical target incision (index procedure), as an adjunct to the SoC treatment. D-PLEX will not be re-administered if any re-intervention occurs.

For subjects randomized to the control arm, the surgical treatment will be as per SoC treatment. SoC will be consistent and standardized for all sites in the clinical study and is composed of IV antibiotic immediately prior to surgery. Each site will use the pre-defined SoC during the study.

For subjects from both arms, preoperative IV antibiotics will be composed from the 1st or 2nd generation of Cephalosporin family plus Metronidazole given within 60 minutes prior to surgery. In cases of allergy to the Cephalosporin or Penicillin families, other IV antibiotic may be used. Pre-operation prophylactic oral antibiotic is not allowed. Mechanical bowel preparation will be at the discretion of the principal investigator (PI) per each site's SOP. Post-operative resumption of activity will be at the discretion of the PI based on the subject's medical condition.

Doxycycline pharmacokinetic sampling will be collected in selected sites from all randomized subjects in these sites (sites from Czech Republic will not participate in the PK sub- study). About [redacted] series of [redacted] samples per set will be analyzed. About [redacted] series will be analyzed from subjects receiving 2 vials, about [redacted] series will be analyzed from subjects receiving 3 vials.

The occurrence of any Adverse Events (AEs), including death, will be recorded throughout the study. All subjects will be followed for an overall of 60 days for safety.

Visit-specific procedures and assessments are outlined in Table 1 below.

Table 1: Study Procedures and Assessments

Procedures	Visit 1 Screening Day -21 to Day 0	Visit 2 Surgery - Day 0	Visit 3 Day 1	Visit 4 Day 5 (± 1 day)	Visit 5 Day 14 (± 3 days)	Visit 6 Day 30 (+ 7 days)	Visit 7 Termination Day 60 (± 7 days)
Informed Consent	X						
Medical History, Charlson Co-morbidity Index & Allergy Questionnaire	X						
General Eligibility Criteria	X	X[1]					
Physical Exam	X					X	X
Vital Signs (blood pressure, heart rate, body temperature)	X	X	X	X	X	X	X
Weight & Height	X						
Pregnancy Test (serum or urine dipstick)[9]	X						
Assessment of Surgical Site[6]			X	X	X[5]	X[5]	X[5]
Blood Tests (hematology, chemistry[3])	X[2]		X	X	X	X	X
Urinalysis [2,4]	X						
Bacteriology test[7]			X[7]	X[7]	X[7]	X[7]	X[7]
Doxycycline PK Sampling[8]		X	X	X	X	X	X
Adverse Events		X	X	X	X[5]	X[5]	X[5]
Concomitant Medications	X	X	X	X	X	X	X
D-PLEX Administration		X					

Notes:

1. Confirmation of Eligibility.

2. May be performed at visit 2 (before surgery).

3. Blood **chemistry and hematology**: for whole list of tests please refer to protocol section 9.1.11. **Coagulation** tests (PT, INR, PTT) only at Screening.

4. **Urinalysis** will be done at screening visit and at the discretion of the Investigator during the other study visits.

5. In case the subject is unable to attend a visit, phone visit should be performed with subject / his community physician / a first degree relative (as per Sponsor's designated form) to obtain information related to subject's safety and wound assessment. Blood tests and vital signs can be collected in the local clinic/laboratory and the results should be send (blinded with subject study number only) to the Sponsor study manager.

6. Surgical site assessment will be performed only if wound is not dressing.

7. Bacteriology test will be performed in case of suspected infection and wound discharge.

8. Blood samples for PK will be collected from about [redacted] subjects at selected sites at the following time-points: [redacted]

9. Pregnancy test should be performed within 1 day before surgery.

3.2. Study Endpoints

3.2.1. Primary Efficacy Endpoint

The primary efficacy endpoint in this study is defined per subject as a binary treatment failure event ("Yes" / "No"), with "Yes" indicating if a subject had experienced within 30 days post abdominal index surgery (up to and including 30 days post index surgery date) any Surgical Site Infection (SSI) event in the target incision, as determined by a blinded and independent adjudication committee. In addition, the following events occurring within 30 days post abdominal index surgery, will be considered as treatment failure events:

- Death (from any reason)
- Re- intervention at the primary incision site (target) due to suspected SSI or due to poor wound healing (including wound dehiscence), as verified by the blinded adjudication committee.

To note, re-intervention at a different access point than the target incision or re-intervention at the target incision site due to any other reason than suspected SSI or poor wound healing (including wound dehiscence), will not be considered as treatment failure event in the primary analysis.

SSI is composed of Deep Incisional Surgical Site Infection (DSSI) and/or Superficial Incisional Surgical Site Infection (SSSI). A SSSI is an infection that involves only skin and subcutaneous tissue of the incision and does not include diagnosis/treatment of cellulitis, a stitch abscess alone or a localized stab wound or pin site infection. A DSSI is an infection that involves deep tissues, such as fascia and muscle layers; this also includes infection involving both superficial and deep incision sites.

Subjects who have a valid non-missing observation of "Day 30 SSI assessment", performed within the pre-specified visit window (applicable visit window is defined below), and have not experienced any adjudicated SSI event or death or any adjudicated re-intervention in the target incision due to suspected SSI or due to poor wound healing, including wound dehiscence, within 30 days post abdominal index surgery, will be defined as "No".

Those subjects who do not have a valid non-missing "Day-30 SSI assessment" as required for the assignment of a Failure='No' response will be considered as having missing data and will be imputed as Failure=Yes for the primary analysis of this endpoint.

For the determination of a 'Failure=No' response, a valid non-missing observation for Day 30 SSI assessment is any confirmation provided in the window of study day [redacted] to study day [redacted] Due to the COVID-19 pandemic, this confirmation can be collected retrospectively through visits performed after study day [redacted]. For an event to be counted as 'Failure=Yes' response, date should be up to and including 30 days post index surgery date.

Details on handling of missing primary endpoint data are described further in section 5.4.

3.2.2. Secondary Efficacy Endpoints

3.2.2.1. Key Secondary Efficacy Endpoints

The key secondary endpoints are defined as follows:

1. Infection rate as measured by the proportion of subjects with at least one SSI event in the target incision, occurred within 30 days post abdominal index surgery, and determined by a blinded independent adjudication committee.

   This endpoint will be defined per subject as a binary ("Yes" / "No") variable, with "Yes" indicating that a subject have had at least one confirmed (by a blinded independent adjudication committee) SSI event within 30 days post index surgery (up to and including 30 days post index surgery date). Subjects who have a valid non-missing observation of "Day 30 SSI assessment", performed within the pre-specified visit window (the same as defined for the primary endpoint), and have not experienced any adjudicated SSI event within 30 days post index surgery, will be defined as "No".

2. Number (percentage) of subjects with at least one score of ASEPSIS > 20 (further to an adjudicated SSI).

   This endpoint will be defined per subject as a binary ("Yes" / "No") variable, with "Yes" indicating that a subject have had at least one score of ASEPSIS > 20. Subjects that have not had a score of ASEPSIS > 20, will be defined as "No".

To note, these endpoints refer to target incision at index surgery. Section 5.4 provides the details on handling missing data here.

3.2.2.2. Additional Secondary Efficacy Endpoints

The current section provides the definitions of the additional secondary efficacy endpoints of the study, general and SSI related. General endpoints will be defined for all subjects in the relevant analysis populations, while SSI related endpoints will be defined only for the sub-population of subjects who experienced at least one adjudicated SSI within 30 days post index surgery.

Unless otherwise noted, and as relevant, endpoints will refer to target incision from index surgery. Section 5.4 provides the details on handling missing data.

The general additional secondary efficacy endpoints are defined as follows:

- Incidence of SSSI within 30 days post index surgery.

  This endpoint will be defined per subject as a binary ("Yes" / "No") variable, with "Yes" indicating that a subject has had at least one confirmed (by a blinded independent adjudication committee) SSSI event within 30 days post index surgery (up to and including 30 days post index surgery date). Subjects who have a valid non-missing observation on Day 30 SSI assessment, performed within the pre-specified visit window (the same as defined for the primary endpoint), and have not

experienced any adjudicated SSSI event within 30 days post index surgery, will be defined as "No".

- Incidence of DSSI within 30 days post index surgery.

  This endpoint will be defined per subject as a binary ("Yes" / "No") variable, with "Yes" indicating that a subject has had at least one confirmed (by a blinded independent adjudication committee) DSSI event within 30 days post index surgery (up to and including 30 days post index surgery date). Subjects who have a valid non-missing observation on Day 30 SSI assessment, performed within the pre-specified visit window (the same as defined for the primary endpoint), and have not experienced any adjudicated DSSI event within 30 days post index surgery, will be defined as "No".

- All-cause mortality rate within 30 days post randomization.

  In case there are no subjects with unknown survival status at 30 days post randomization, this endpoint will be defined per subject as a binary ("Yes" / "No") variable, with "Yes" indicating that a subject had died (from any reason) within 30 days post randomization. Otherwise, the 30-days mortality rate will be estimated using a time-to-event analysis, by the Kaplan-Meier method. In this case, for each subject survival time will be calculated as days between randomization and death (in the event of death within 30-days) or the time from randomization to the earliest between last date of known to be alive or 30-days, yielding a censored survival time.

- All-cause mortality rate within 60 days post randomization.

  This endpoint will be defined similarly to the 30 days mortality endpoint above, now with the time frame of 60 days post randomization.

- Time to adjudicated SSI within 30 days post index surgery.

  This endpoint will be defined per subject as the difference between the date of adjudicated SSI and the date of index surgery. Subjects, who have not experienced an adjudicated SSI within 30 days post index surgery, will be handled as follows:

  - Subjects having a valid non-missing Day 30 SSI assessment (within the pre-specified visit window) will be censored at Day 30.
  - Subjects who died within 30 days post index surgery will be censored at the date of death.
  - Subjects having a missing Day 30 SSI assessment (or performed out of the pre-specified visit window) will be censored at the date of the last valid SSI assessment, performed within 30 days post index surgery.
  - Subjects having no valid SSI assessment within 30 days post index surgery will be censored at the date of index surgery (i.e. at Day 0).

- Number (percentage) of subjects, re-admitted within 30 days post index surgery (for any reason) and experienced adjudicated SSI during this re-admission.

This endpoint will be defined per subject as a binary ("Yes" / "No") variable, with "Yes" indicating that a subject had been re-admitted within 30 days post index surgery (for any reason) and experienced an adjudicated SSI during this re-admission. Subjects who have a valid non-missing observation on Day 30 SSI assessment, performed within the pre-specified visit window (the same as defined for the primary endpoint), and have not been re-admitted within 30 days post index surgery (for any reason) with an adjudicated SSI during this re-admission, will be defined as "No".

- Number (percentage) of subjects who experienced at least one adjudicated surgical re-intervention within 30 days post index surgery.

  This endpoint will be defined per subject as a binary ("Yes" / "No") variable, with "Yes" indicating that a subject had experienced at least one adjudicated surgical re-intervention within 30 days post index surgery. Re-intervention is defined as re-opening of the surgery incision, used for the original index surgery, in the operation room (OR). Adjudicated re-intervention is re-intervention due to suspected SSI or due to poor wound healing (including wound dehiscence), as verified by the blinded adjudication committee. Subjects who have a valid non-missing observation on Day 30 SSI assessment, performed within the pre-specified visit window (the same as defined for the primary endpoint), and have not experienced any adjudicated re-intervention within 30 days post index surgery, will be defined as "No".

- Number (percent) of subjects who experienced at least 1 surgical re-intervention during 30 days post index surgery.

  This endpoint will be defined as the previous endpoint, where now any re-intervention (regardless of reason) will be considered for Yes outcome.

The SSI related additional secondary efficacy endpoints will be applied only for subjects with adjudicated SSI within 30 days post index surgery and are defined as follows:

- Number (percentage) of subjects that at least one bacteria resistant to Doxycycline was isolated during the bacteriological tests.

  This endpoint will be defined per subject as a binary ("Yes" / "No") variable, with "Yes" indicating that a subject had at least one bacteria (grown during the bacteriological tests) which is resistant to Doxycycline.

- Number (percentage) of bacteria resistant to Doxycycline out of all bacteria grown during the bacteriological tests.

  This endpoint will be defined per unique bacteria (grown during the bacteriological tests) per subject as a binary ("Yes" / "No") variable, with "Yes" indicating that a unique bacteria is resistant to Doxycycline.

- Number (percentage) of subjects who received antibiotic (any route) as a treatment for their adjudicated SSI.

This endpoint will be defined per subject as a binary ("Yes" / "No") variable, with "Yes" indicating that a subject received antibiotic as a treatment for their adjudicated SSI.

- Number of IV antibiotic treatment days.

  This endpoint will be defined per subject as the total number of days on which a subject was treated with IV antibiotic. Subjects who were not treated with IV antibiotic will be assigned a value of 0 days.

- Average of subjects' cumulative ASEPSIS assessment score (AUC).

  This endpoint will be defined per subject as Area Under the Curve (AUC) of ASEPSIS assessment scores, calculated using the trapezoidal rule, in the time frame of 60 days from index surgery. This endpoint will be calculated to provide a measure of total burden (for example, for two patients reaching the same maximal score, a patient whose score applied for a larger duration will be scored higher than one with shorter duration).

- Number (percentage) of subjects with at least one score of ASEPSIS > 20.

  This endpoint will be defined per subject in the same way as the second key secondary efficacy endpoint (refer to section 3.2.2.1, item 2), and will apply here only for sub-population of subjects with adjudicated SSI within 30 days post index surgery.

3.2.3. Safety Endpoints

The following safety endpoints will be evaluated:

- Adverse events
- Physical examination
- Vital signs
- Incisional wound healing
- Laboratory tests

In addition, concomitant medications, surgery and hospitalization characteristics are collected.

The definitions and analyses of these safety measures are provided in section 8 of this SAP.

3.3. Sample Size and Power Considerations

3.3.1. The Minimal Sample Size Calculation

The sample size calculation for this study is based on powering the study with respect to the primary efficacy analysis. The treatment failure rates were compared between the two treatment groups: D-PLEX + Standard of Care (SoC) versus SoC alone.

Assumptions for the sample size calculations were as follows:

- Test: two-sample proportion difference using chi-square test
- Significance level: two-sided $\alpha = 0.04$, taken conservatively to account for the planned interim analysis for efficacy (to be performed with a two-sided $\alpha=0.01$, refer to section 5.3 for full details).
- Power = 90%
- Null hypothesis: $P_{SoC}=P_{D\text{-}PLEX+SoC}$
- Allocation ratio: 1:1
- SoC SSI rate: 16%
- Effect size of D-PLEX+SoC on SSI rate, compared to SoC alone: 50% (halving the SSI rate), therefore, the assumed D-PLEX+SoC SSI rate was 8%
- All-cause Death or Adjudicated Re-intervention in the target incision due to suspected SSI or due to poor wound healing, including wound dehiscence, rate: [redacted] for both treatment groups, therefore, the assumed treatment failure rates were [redacted] and [redacted], for SoC and D-PLEX+SoC, respectively

Based on the above assumptions, a sample of 882 subjects provide 90% power to detect the assumed difference in treatment failure proportions between the treatment groups. In order to account for an anticipated of about 5% lost-to-follow-up rate, a minimum of 950 subjects (475 per treatment group) will be enrolled.

3.3.2. Interim Analysis for Stopping Early for Efficacy or Futility, or Unblinded Sample Size Re-estimation

One comparative interim analysis for an early efficacy or futility stop or unblinded sample size re-estimation will be conducted when about 750 subjects complete one month follow-up and are evaluated for the primary endpoint. The interim analysis will include a group-sequential design stopping rule for efficacy or futility, combined with unblinded sample size re-assessment based on the 'Promising Zone' approach. The outcome of this interim analysis will be either early stopping for efficacy or futility, or continuation to planned final (950 subjects) or continue with sample size increased up to total of 1400 subjects only if the comparative result falls in a predefined 'Promising Zone'. The interim decision will be made by the independent DMC according to a pre-planned interim analysis plan, and

comparative information will be maintained confidential within the independent statistician until the study finally unblinded.

The full details about the stopping rules and the sample size decision rules will be detailed in an Interim Statistical Analysis Plan (ISAP) accompanying this document.

3.4. Randomization and Blinding

3.4.1. Randomization

Upon confirmation that the subject fulfils all inclusion criteria and none of the exclusion criteria are met, he/she will be randomized through an Interactive Web Randomization System (IWRS) integrated with the electronic Case Report Form (eCRF). Randomization will be stratified by type of prophylactic Standard of Care (prophylactic IV antibiotic with mechanical bowel preparation or prophylactic IV antibiotic without mechanical bowel preparation) and by region (USA or Europe+Israel).

Randomization will be done centrally, using a randomization specification prepared by the study statistician and generated by an independent statistician (not involved in the study), and will assign subjects in a 1:1 ratio to:

- Treatment Arm – Standard of Care (SoC) concurrently with D-PLEX application
- Control Arm – SoC treatment alone

Instructions and training for randomization and stratification process will be provided during site initiation visit.

The randomization code will be stored within the IWRS database until unblinding of this study is requested.

3.4.2. Blinding

This is a double blind clinical trial. The sponsor, subjects, outcome assessor and all staff involved in the collection and recording of the clinical and laboratory data, based on which the independent adjudication committee will perform their assessment, will be blind to treatment assignment. In addition, all aspects of data management and clean-up as well as programming for final analysis will be done in blinded datasets. An unblinded statistician, external to the Sponsor, will be tasked with providing unblinded data to the Data Monitoring Committee (DMC) and will have access to the treatment codes via the Interactive Web Response System (IWRS).

The study site personnel, who perform the index surgery (operation room staff), will be trained not to disclose the treatment arm to the blinded investigator, to the subject, his/her family, to other health care providers not present during the surgery or to the study Sponsor representatives.

Wound assessment and other study outcomes throughout the study follow-up visits will be done by a blinded investigator that will not be involved in the surgery.

3.5. Sequence of Planned Analyses

3.5.1. Interim Analyses

One formal interim analysis to stop the study early for efficacy or futility, or sample size re-estimation is planned, scheduled to be conducted when about 750 subjects will complete the 1-month follow-up and evaluated for the primary endpoint. The interim analysis will include a group-sequential design stopping rule for efficacy or futility, combined with unblinded comparative sample size re-estimation based on the 'Promising Zone' approach (Mehta and Pocock 2011). Only the primary efficacy endpoint will be analysed.

The outcome of this interim analysis will be either early stopping for efficacy or futility, or continuation to planned final (950 subjects) or continue with sample size increased up to maximal size of 1400 only if the comparative result falls in a predefined 'Promising Zone'. As specified in section 3.3.2, the full details about the stopping rules and the sample size decision rules will be detailed in an Interim Statistical Analysis Plan (ISAP) accompanying this document.

3.5.2. Final Analyses and Reporting

All final, planned analyses identified in this SAP will be performed only after the last randomized subject has completed the study. For the purpose of the interim analysis, the randomization codes will not be unblinded until the ISAP has been signed and approved and accordingly prior to final analysis, the randomization codes will not be unblinded until this document has been signed and approved.

Any exploratory analyses completed to support study analyses, which were not identified in this SAP, will be documented and reported in appendices to the CSR.

4. ANALYSIS POPULATIONS

The current section describes the analysis populations defined for the study.

4.1. Screened

This population will include all subjects who underwent screening and will be used for summary of disposition and data listings.

4.2. Intent to Treat (ITT)

The ITT population will include all abdominally-incision randomized subjects. In this population, treatment will be assigned based upon the treatment to which subjects were randomized regardless of which treatment they actually received.

4.3. Safety

The safety population will include all subjects randomized and treated with D-PLEX or SoC. In this population, treatment will be assigned based upon the treatment subjects actually received regardless of the treatment to which they were randomized.

4.4. Modified Intent to Treat (mITT)

The mITT analysis population is a sub-population of the ITT and will include all subjects randomized and treated with D-PLEX or SoC, who underwent the index surgery and have no eligibility criteria deviation. Exclusion from the mITT population will be determined in a blinded manner prior to database lock. In this population, treatment will be assigned based upon the treatment to which subjects were randomized regardless of which treatment they actually received. Subjects who will die during the study will not be excluded from the mITT population.

4.5. Per Protocol (PP)

The PP analysis population will include all mITT subjects who had no major protocol deviations and assessed for primary endpoint (i.e. had Day 30 SSI assessment within the pre-specified visit window, or had been defined as treatment failure before this assessment due to experiencing an adjudicated SSI event, or death, or adjudicated re-intervention on the target incision due to suspected SSI or due to poor wound healing, including wound dehiscence). In this population, treatment will be assigned based upon the treatment to which subjects were randomized regardless of which treatment they actually received.

5. GENERAL ASPECTS FOR DATA ANALYSIS

5.1. General

The following is a list of general reporting conventions:

1. Descriptive statistics for continuous variables include n (number of non-missing observations), mean, standard deviation (SD), standard error of the mean (SE), 25th and 75th percentiles, median, minimum, and maximum.
2. Means, medians and percentiles will be reported with 1 more digit than the precision of the captured data. Standard deviations and confidence intervals will be reported at 2 more digits than the precision of the data. Minimum and maximum will be reported to the same level of precision as the original observations.
3. Descriptive statistics for categorical variables will include counts (n) and percentages (%) and will be presented in the form of n (%).
4. Percentages will be reported with 1 decimal place.
5. No preliminary rounding should be performed; rounding should only occur after analysis. To round, consider digit to right of last significant digit: if < 5, then round down; if ≥ 5, then round up.
6. Unless otherwise noted, all statistical analyses will be conducted with a significance level (α) of 0.05 and utilize two-sided testing.
7. All data collected will be presented in the by-subject data listings, sorted by subject and by time point, where appropriate.

If departures from these general conventions are present in the specific evaluation sections of this SAP, then those will take precedence over the above general conventions.

5.2. Specification of Baseline Values

In general, Baseline will be defined for each subject as the last available, valid, non-missing assessment before the initiation of first study treatment, i.e. before the administration of pre-operative IV antibiotic, given as part of Standard of Care procedures.

5.3. Multiple Comparisons and Multiplicity

The overall study-wise type I error will be 5%. To protect the study from type I error inflation, the key secondary endpoints will be interpreted inferentially only if a statistically significant treatment effect is detected in the primary endpoint. To note, as the secondary endpoints are to be tested once (not part of interim analysis), the first secondary endpoint will be tested in full 5% significance level in case the primary endpoint is successful. Type I error will be further controlled for the key secondary endpoints by employing the Hierarchical Approach, i.e., the second key secondary endpoint will be formally analysed

only in case the preceding first key secondary endpoint is statistically significant (p-value ≤ 0.05).

The additional secondary endpoints will be used to enhance understanding the beneficial effect of D-PLEX and there is no plan for formal inference for these. Thus, no multiplicity control is further planned beyond the key secondary endpoints.

To account for the option of early stopping for efficacy, the type-1 error spending approach will be utilized. The interim analysis is planned when about 750 subjects in total will be enrolled and have the chance to complete 1-month visit, and an alpha level of 1% (two-sided) regardless of the exact number of subjects included in the analysis will be used. *For strict control of the overall type-1 error, the alpha for the final analysis (in case the study does not stop at interim) will be calculated based on the actual information time* of the interim analysis (actual total # at interim/target maximal study size).

As the interim analysis was performed with a total of [redacted] patients and the final study size remained un-changed at 950 subjects, the calculated one-sided alpha level for the primary endpoint to maintain overall of 5% significance level is: [redacted] ([redacted] one sided, [redacted] two sided).

The 'Promising Zone' approach (Mehta and Pocock 2011) was chosen to rigidly control study overall type-1 error due to the unblinded comparative size re-estimation at the time of interim analysis, and its planned implementation strictly follows the rules that increase sample size only if comparative results fall in a pre-defined 'Promising Zone', thus allowing final analysis to be preserved without further need for statistical adjustment (beyond adjustment due to the interim efficacy analysis as described above).

5.4. Handling Withdrawals and Missing Data

5.4.1. Primary Endpoint

To be recalled, in the calculation of the primary endpoint (section 3.2.1), deaths occurring within the first 30 days post abdominal index surgery are addressed as treatment failure events and thus are not considered missing data problem, in either of the primary or sensitivity and supportive primary endpoint analysis.

To note, re-intervention in the target incision occurring within the first 30 days post abdominal index surgery due to suspected SSI or due to poor wound healing (including wound dehiscence), as verified by the blinded adjudication committee, will be addressed as treatment failure events ("Yes") in all sensitivity and supportive analyses of the primary efficacy endpoint.

As specified in section 3.2.1, for the purpose of the primary efficacy analysis, based on ITT population, missing data will be imputed as follows: Subjects, who have missing primary efficacy endpoint data due to other reasons than death (i.e. missing Day 30 SSI assessment (regardless of reason) or performed out of pre-specified visit window) and have not already been identified as treatment failures due to having experienced any adjudicated SSI event

or any adjudicated re-intervention in the target incision, due to suspected SSI or due to poor wound healing, including wound dehiscence, will be imputed as treatment failures ("Yes").

As defined in section 3.2.13.2.1, for the determination of a 'Failure=No' response, a valid non-missing observation for Day 30 SSI assessment is any confirmation of such response provided in the window of study day [redacted] to study day [redacted] Due to the COVID-19 pandemic, this confirmation can be collected retrospectively through visits performed after study day [redacted].

For the purpose of the sensitivity analyses to the primary efficacy endpoint analysis, missing data (arising from other reasons than death) will be imputed as follows:

When using ITT population:

1. Using Multiple Imputation (MI) technique
2. Imputing as not treatment failure ("No")

The MI technique will be performed in 3 steps:

1. Step 1- Imputation: missing data will be filled in using 50 different sets of values, to produce 50 imputed datasets. The imputed datasets will consist of 50 copies of the original ITT set, with the observed values being the same across all copies, and with imputed values varying from one copy to another. The logistic regression method as implemented in the MI procedure in SAS (the MONOTONE LOGISTIC statement) will be used for this step. The variables that will be included in the imputation model will be as follows: treatment group, stratification factors, age, gender and incisional length of > 20 cm (Yes/No). In case of complete separation issues, the baseline factors list (except treatment) may be reduced.

2. Step 2- Analysis: each datasets will be analyzed separately using the same analytical approach defined for the primary analysis (namely, the CMH test).

3. Step 3- Pooling: The results from the 50 imputed datasets will be combined into one result using Rubin's rules which account for the uncertainty associated with imputed values. This pooling will be performed using the MIANALYZE procedure in SAS. A normalizing Wilson-Hilferty transformation will be applied to the CMH statistic and the resulted normal variable will be further standardized prior to pooling, following the method proposed in Ratitch et al.

When using mITT and PP populations: no imputation will be performed (i.e., a complete case analysis will be used).

5.4.2. Secondary Endpoints

There will be no imputation of missing data for all other study endpoints (i.e., a complete case analysis will be used), unless otherwise noted for a specific analysis.

Specifically for the key secondary efficacy endpoints, sensitivity analysis for missing data due to reasons other than deaths may be performed, depending on the number of these cases, using the same methods specified above for the primary endpoint.

There is no planned sensitivity analysis for missing data in other secondary endpoints.

Time to adjudicated SSI within 30 days post index surgery endpoint will handle the missing values as censored, as detailed in the definition of this endpoint in section 3.2.2.2.

Details on imputation of partial dates for concomitant medications and for adverse events are provided further in sections 8.7.1 and 8.2, respectively.

5.5. Study days and visit windows

The day of initiation of study treatment in this study is defined as the day of index surgery (Day 0). Study days will be numbered relative to the day of index surgery (i.e. …, –2, –1, 0, 1, 2, …), with day –1 being the day before index surgery, day 0 being the day of index surgery, day 1 being 1 day post index surgery.

In general, the study data will be summarized as collected at the scheduled study visits regardless of visit window violations. For by-visit summaries, if there are multiple assessments at a post-baseline visit, then the last non-missing assessment at that visit will be used for the summary.

For the primary efficacy endpoint definition, the pre-specified analysis-visit window for Day 30 follow-up visit will be used, as described in section 3.2.1 above.

6. STUDY POPULATION

6.1. General

Study population summaries will be presented by treatment group and overall unless otherwise noted. Subject disposition will include all screened subjects. All other study population summaries will be based on ITT population unless otherwise noted.

6.2. Subject Disposition

The subject disposition will be summarized as follows and presented for each treatment group and overall, unless otherwise specified. The percentages will be calculated from the number of randomized subjects, unless otherwise specified.

- The number of all screened subjects (i.e. the number of subjects in the Screened population), presented only for overall group
- The number (%) of subjects who failed screening (% calculated from the Screened population), including the distribution of reasons for failing the screening, presented only for overall group
- The number (%) of subjects screened but not randomized, if applicable (% calculated from the Screened population), presented only for overall group
- The number of randomized subjects
- The number (%) of randomized but not treated subjects, if applicable
- The number (%) of subjects in the Safety population (i.e. treated subjects)
- The number (%) of subjects in the ITT population
- The number (%) of subjects in the mITT population (% calculated from ITT population)
- The number (%) of subjects in the PP population (% calculated from ITT population)
- The number (%) of subjects who completed the study
- The number (%) of subjects who discontinued the study prematurely, including the distribution of reasons for premature discontinuation

6.3. Demographics and Baseline Characteristics

Demographics and baseline characteristics will be summarized by treatment group and overall using ITT and Safety populations. Summaries will be presented with the appropriate descriptive statistics as specified in section 5.1 above.

The following will be provided:

- Demographics:
  - Age (years) (continuous)
  - Gender: Male, Female (categorical)
  - Childbearing Potential: Yes, No (categorical, females only)
  - Ethnicity: Hispanic or Latino, Not Hispanic or Latino, Unknown, Not Stated (categorical)
  - Race: White, American Indian or Alaska native, Asian, Black or African American, Native Hawaiian or Other Pacific Islander, Other (categorical). As a subject may chose more than one categories, combinations will also be presented, such as a patient will contribute to only single category.
- Baseline vital signs:
  - Height (cm) (continuous)
  - Weight (kg) (continuous)
  - BMI (kg/m^2) (continuous)
- Allergy History:
  - Any known allergy: Yes, No (categorical)
  - Any known allergy to Doxycycline and/or to the tetracycline family of drugs: Yes, No (categorical)
  - Any hospitalization due to allergy reaction: Yes, No (categorical)
  - Taking antihistamine drugs in the last year for any reason: Yes, No (categorical)
  - Suffering from allergic type symptoms: Yes, No (categorical)
- Substance Use (only subjects for whom Substance Use data had been collected):
  - Cigarettes (Tobacco):
    - Usage: Current, Former, Never (categorical)
    - Number of Pack-Years (for current or former usage) (continuous)
  - Alcohol:
    - Usage: Current, Former, Never (categorical)
    - Frequency: Daily, Occasionally, Almost Never (for current or former usage) (categorical)
  - Recreational Drugs Abuse:
    - Usage: Current, Former, Never (categorical)

  - Frequency: Daily, Occasionally, Almost Never (for current or former usage) (categorical)
  - Drug Type: Amphetamines, Barbiturates, Benzodiazepines, Cocaine, Methadone, Methamphetamines, Opiate, Phencyclidine, Tricyclic Antidepressants, Cannabinoids, Other, Unknown/Not reported (categorical)
- Charlson Co-Morbidity Index (CCI), total score
- Is this a COVID-19 patient: Confirmed Positive, Suspected, Unlikely, Confirmed Negative, Not Done.
- Indication for Index Surgery: Cancer, Crohn's Disease, Inflammatory Bowel Disease (IBD), Other (categorical)

The following baseline factors are identified as prognostic factors that may be associated with primary outcome for which group balance will be reviewed: age, gender, comorbid conditions (Yes/No) associated with increased risk of SSI (as listed in section 6.5), Charlson co-morbidity index, indication for index surgery. If any of these baseline factors are deemed imbalanced then the factor will be included as an extra adjusting covariate in the relevant sensitivity analysis models for the primary and key secondary efficacy endpoints.

6.4. Stratification Factors for Randomization

Stratification factors for randomization will be summarized by treatment group and overall using ITT population. Summaries will be presented with the appropriate descriptive statistics as specified in section 5.1 above.

The following will be provided:

- Type of Prophylactic Standard of Care: IV antibiotics With Mechanical Bowel Preparation, IV antibiotics Without Mechanical Bowel Preparation (categorical)
- Region: USA, Europe+ Israel (categorical)

To note, errors in randomization (selecting the wrong category of the factor) are not possible for the Region factor, however, are possible for the type of prophylactic SoC factor. If these happen, the corrected factor (as presented in the CRF) will be presented as well.

6.5. Medical History

Medical history data will be summarized by treatment group and overall using Safety population. All medical history terms will be coded using the Medical Dictionary for Regulatory Activities (MedDRA, version 23.1). The number and percentage of subjects with at least one medical history term will be summarized by System Organ Class (SOC)

and by Preferred Term (PT) within SOC for each treatment group and overall. The number of events will also be summarized. The table will be sorted by overall descending frequency of SOC and then, within a SOC, by overall descending frequency of PT.

Additional summary table will be provided for the following comorbid conditions (associated with increased risk of SSI): Diabetes, Chronic Obstructive Pulmonary Disease (COPD)/Smoking, Obesity/Overweight, Hypertension, and Peripheral Vascular Disease (PVD). The number and percentage of subjects will be presented for each comorbid condition by treatment group and overall.

All medical history data will be listed.

6.6. Physical Examination

Physical examination findings at baseline (Normal, Abnormal CS, Abnormal NCS, Missing) will be summarized by treatment group and overall using Safety population. Summaries will be presented with the appropriate descriptive statistics as specified in section 5.1 above.

6.7. Protocol Deviations

All protocol deviations in the study will be listed. Summary of deviations according to pre-defined categories will also be provided by treatment group and overall.

7. EFFICACY ANALYSES

7.1. General

The analysis of the primary and key secondary efficacy endpoints will be performed on the ITT, mITT and PP populations, with the ITT population serving as the primary analysis population. General additional secondary efficacy endpoints will be analysed using the ITT and mITT populations, unless otherwise noted for a specific endpoint. SSI related additional secondary efficacy endpoints will be analysed on the sub-population of subjects who experienced at least one adjudicated SSI within 30 days post index surgery.

Summaries will be presented by treatment group, unless otherwise noted.

7.2. Primary Efficacy Analysis

The primary objective of the study is to assess the anti-infective efficacy of DPLEX+SOC over a period of 30 days post operation, by preventing surgical site infection (SSI), defined as superficial and/or deep infection, in the index (target) incision, compared to the SoC alone control arm.

7.2.1. Primary Analysis

The primary efficacy analysis will be based on the ITT population and will compare the proportions of treatment failures (as defined in section 3.2.1 above) between D-PLEX+SoC and SoC alone treatment groups.

The number and percentage of subjects, defined as treatment failures, will be presented by treatment group. A 95% confidence interval (based on the Clopper-Pearson method) will be constructed for each proportion. A Cochran–Mantel–Haenszel (CMH) test will compare the proportions of treatment failure between the two groups, using the study stratification factors used for randomization (as specified in section 3.4.1 above), and corresponding risk difference estimate will be presented with 95% confidence interval (using the method of Mantel–Haenszel stratum weights, as applied in the COMMONRISKDIFF option in the SAS FREQ procedure). The following generic SAS syntax will be used:

```
ODS OUTPUT CommonPdiff=cmh_pdiff(WHERE=(method="Mantel-Haenszel"))
           CMH=cmh_pval(WHERE=(AltHypothesis="Row Mean Scores Differ"));
PROC FREQ DATA=eff ORDER=internal;
TABLES STRATAA*STRATAB*TRTP*AVALC / CMH COMMONRISKDIFF(COLUMN=2);
RUN;
```

The following hypotheses will be tested for a significant difference in the treatment-failure proportions between D-PLEX+SOC (P_{DPLEX}) and SoC alone (P_{SoC}) treatments:

$$H_0: P_{DPLEX} = P_{SoC}$$

vs.

$$H_1: P_{DPLEX} \neq P_{SoC}$$

For final analysis, the interim analysis adjusted significance level will be used for this test, refer to section 5.35.3 for details. In the case of small number of events (less than 5 events in any treatment group), the Fisher's exact test will be used.

In addition, the number and percentage of each of the failure types comprising the primary efficacy endpoint will be presented by treatment group. The denominator for percentages will be the number of ITT subjects in each treatment group. The mutually exclusive failure type categories are:

1. Death
    1.1. Death and Adjudicated DSSI Event
    1.2. Death and Adjudicated SSSI Event
    1.3. Death and Adjudicated Re-intervention
    1.4. Death, without Adjudicated SSI Event or Adjudicated Re-intervention
2. Adjudicated SSI Event (Total)
    2.1. Adjudicated DSSI Event
    2.2. Adjudicated SSSI Event
3. Adjudicated Re-intervention
4. Missing Day 30 SSI Assessment.

Subjects experiencing more than one type of treatment failure event will be counted only in the highest in the hierarchy type (based on the above order). To note, adjudicated DSSI and SSSI events, all-cause mortality, and adjudicated re-interventions will be analysed further as a part of the additional secondary efficacy analyses.

7.2.2. Sensitivity and Supportive Analyses

Sensitivity and supportive analyses for the primary endpoint analysis will address the following aspects:

1) Repeat the primary analysis using the mITT and PP populations.
2) To account for the possibility of errors in values of stratification factors used for randomization, the primary analysis will supportively be repeated using the correct values.
3) Difference between the treatment groups when controlling for possible imbalance in important baseline factors (as obtained from the analyses described in

section 6.3) will be analysed by evaluating the odds ratio obtained from estimating a multiple logistic regression model.

4) Sensitivity analyses for the imputation of missing data due to other reasons than death (please refer to section 5.45.4.1).

5) Descriptive statistics of the incidence of re-interventions at the target incision site due to other reasons than suspected SSI or due to poor wound healing (including wound dehiscence), within 30 days post abdominal index surgery. Sensitivity analysis where these events are considered also as treatment failure (in addition to the primary endpoint treatment failure events, as detailed in section 3.2.1) will also be performed.

All analyses of the primary endpoint are based on the blinded and independent adjudication committee decisions. The investigator assessment of SSI will be described and listed, as detailed in section 8.5.

7.3. Secondary Efficacy Analyses

7.3.1. Key Secondary Efficacy Analyses

7.3.1.1. First Key Secondary: SSI Rate within 30 Days Post Index Surgery

The number and percentage of subjects with at least one SSI event (as defined in section 3.2.2.1, item 1) will be presented by treatment group. A 95% confidence interval (based on the Clopper-Pearson method) will be constructed for each proportion. A Cochran–Mantel–Haenszel (CMH) test will compare the proportions of subjects with at least one SSI event between the two groups, using the study stratification factors used for randomization (as specified in section 3.4.1 above), and corresponding risk difference estimate will be presented with 95% confidence interval (using the method of Mantel–Haenszel stratum weights, as applied in the COMMONRISKDIFF option in the SAS FREQ procedure, generic SAS syntax detailed in section 7.2.1 above). In the case of small number of events (less than 5 events in any treatment group), the Fisher's exact test will be used.

This first key secondary efficacy endpoint will be formally tested only if the primary efficacy endpoint analysis showed statistically significant difference between the groups in the favor of D-PLEX+SoC, using two-sided 5% significance level (per section 5.3).

7.3.1.2. Second Key Secondary: ASEPSIS>20 Rate

The second key secondary efficacy endpoint (as defined in section 3.2.2.1, item 2) will be analysed using the same methods as described for the first key secondary efficacy endpoint (refer to section 7.3.1.1 above).

This second key secondary efficacy endpoint will be formally tested only if the first key secondary efficacy endpoint showed statistically significant difference between the groups in the favor of D-PLEX+SoC, using two-sided 5% significance level.

The same sensitivity analyses as defined for the primary endpoint will be performed for the two key secondary efficacy endpoints. Sensitivity analyses for missing data in these endpoints are described in section 5.4.2.

7.3.2. Additional Secondary Efficacy Analyses

The current section describes the analyses to be performed on the additional secondary efficacy endpoints, as defined in section 3.2.2.2.

General additional secondary efficacy endpoints will be analysed using the ITT and mITT populations, unless otherwise noted for a specific endpoint. No other sensitivity analyses are planned for these endpoints. The following analyses will be provided:

- Incidence of SSSI within 30 days post index surgery.

  This endpoint will be analysed using the same methods as described for the first key secondary efficacy endpoint (refer to section 7.3.1.1 above).

- Incidence of DSSI within 30 days post index surgery.

  This endpoint will be analysed using the same methods as described for the first key secondary efficacy endpoint (refer to section 7.3.1.1 above).

- All-cause mortality rate within 30 days post randomization.

  In case there are no subjects with unknown survival status at 30 days post randomization, this endpoint will be analysed using the same methods as described for the first key secondary efficacy endpoint (refer to section 7.3.1.1 above). Otherwise, time to event survival analysis methods will be employed in which subjects with unknown survival status will be censored on the last date of known to be alive. In this later case, Day 30 mortality rates, along with 95% confidence intervals, will be estimated from Kaplan-Meier curves and Z test will be used to compare these rates between the treatment groups.

- All-cause mortality rate within 60 days post randomization.

  This endpoint will be analysed using the same methods as described above for the all-cause mortality rate within 30 days post randomization endpoint, while changing 30 days to 60 days.

- Time to adjudicated SSI within 30 days post index surgery.

  Time to adjudicated SSI will be estimated using Kaplan-Meier (KM) method. A graphical representation of the data will be done using KM curves. The corresponding restricted mean times (by day 30) with 95% confidence intervals will

be estimated. In addition, the number of censored observations will be provided by treatment group. The treatment groups will be compared with a stratified log rank test, using the study stratification factors used for randomization (as specified in section 3.4.1 above). In addition, cumulative incidence plot, treating death as competing event, will be provided.

- Number (percentage) of subjects, re-admitted within 30 days post index surgery (for any reason) and experienced adjudicated SSI during this re-admission.

  This endpoint will be analysed using the same methods as described for the first key secondary efficacy endpoint (refer to section 7.3.1.1 above).

- Number (percentage) of subjects who experienced at least one adjudicated surgical re-intervention within 30 days post index surgery.

  This endpoint will be analysed using the same methods as described for the first key secondary efficacy endpoint (refer to section 7.3.1.1 above).

- Number (percent) of subjects who experienced at least 1 surgical re-intervention during 30 days post index surgery.

  This endpoint will be analysed using the same methods as described for the first key secondary efficacy endpoint (refer to section 7.3.1.1 above).

SSI related additional secondary efficacy endpoints will be analysed on the sub-population of subjects who experienced at least one adjudicated SSI within 30 days post index surgery. The following analyses will be provided:

- Number (percentage) of subjects who had at least one bacteria resistant to Doxycycline grown during the bacteriological tests.

  This endpoint will be tabulated by treatment group, presenting the number and percentage of subjects in each category ("Yes" / "No"). Chi square test (or Fisher's exact test, as applicable) will be used to compare the treatment groups, and corresponding risk difference estimate will be presented with 95% confidence interval.

- Number (percentage) of bacteria resistant to Doxycycline out of all bacteria grown during the bacteriological tests.

  This endpoint will be tabulated by treatment group, presenting the number and percentage of bacteria in each category ("Yes" / "No").

- Number (percentage) of subjects who received IV antibiotic as a treatment for their adjudicated SSI.

  This endpoint will be analysed using the same methods as described above for the number (percentage) of subjects who had at least one bacteria resistant to Doxycycline grown during the bacteriological tests endpoint.

- Number of IV antibiotic treatment days.

This endpoint will be summarized by treatment group, using the appropriate descriptive statistics as specified in section 5.1 above. A Wilcoxon Rank Sum Test will be conducted to assess for treatment group differences.

- Average of subjects' cumulative ASEPSIS assessment score (AUC).

  This endpoint will be summarized by treatment group, using the appropriate descriptive statistics as specified in section 5.1 above. A Wilcoxon Rank Sum Test will be conducted to assess for treatment group differences.

- Number (percentage) of subjects with at least one score of ASEPSIS > 20.

  This endpoint will be analysed using the same methods as described above for the number (percentage) of subjects who had at least one bacteria resistant to Doxycycline grown during the bacteriological tests endpoint.

Bacteria- related additional descriptive analysis:

- Number (percentage) of bacteria resistant to any antibiotics (any, exactly 1, exactly 2, exactly 3 and more than 3 antibiotics) out of all bacteria grown during the first and all bacteriological tests based on the safety analysis population (regardless of confirmed SSI status).

7.4. Sub-group Analysis

The primary and key secondary efficacy endpoints will be analysed by incision length with the following categories: ">10 – ≤ 20 cm" and "> 20 cm". Subgroup analyses will be performed using the same methods specified for the primary and key secondary analyses.

The primary and the first key secondary efficacy endpoints will also be analysed by mechanical bowel preparation received ("Yes/No"). As mechanical bowel preparation is a stratification variable, crude analyses will be applied in each subgroup.

8. SAFETY ANALYSES

8.1. General

Safety analyses will be based on Safety population, unless otherwise noted. All summaries will be presented by treatment group and overall, unless otherwise noted.

8.2. Adverse Events

All adverse events (AEs) will be coded using the Medical Dictionary for Regulatory Activities (MedDRA, version 23.1). Only Treatment Emergent Adverse Events (TEAEs) will be summarized. TEAEs are defined as all AEs that start on or after the date of the index surgery. If the AE start date is incomplete, unless it is clear that the date is prior to index surgery, it will be conservatively imputed as follows for the purpose of determining TEAE flag:

- If the start date is completely missing, the start date will be equal to the date of the index surgery. However, if the stop date is not missing and is before the date of the index surgery, then the stop date will be used instead.
- If the start day is missing, but the month and year are not missing and are equal to the month and year of the index surgery, then the day of the index surgery will be used.
- If the start day is missing, but the month and year are not missing and are not equal to the month and year of the index surgery, then the first day of the month will be used.
- If the start day and month are missing, then the day and the month of the index surgery will be used.

Please note, AE summary tables will include also all suspected SSI Events, reported by investigator on Suspected SSI Events form.

Every AE will be graded by the investigator as mild, moderate, or severe and will be assessed by the investigator for its relationship to the IP (related, possibly related, unlikely related, not related), and for relationship to other causes (concurrent illness, surgical intervention, other, none). AEs assessed for relationship to the IP as "Unlikely Related" or "Not Related" will be summarized under "Not Related" category; AEs assessed for relationship to the IP as "Related" or "Possibly Related" will be summarized under "Related" category; AEs with missing assessment for relationship to the IP will be conservatively included in "Related" category.

When reporting by System Organ Class (SOC) and PT, the reports will present the SOC in alphabetical order; while PTs within the SOC will be presented in order of overall decreasing frequency of occurrence. A patient with multiple AEs (different PTs) coded to the same SOC will be counted only once for that SOC, but will be counted each time for

different PTs within that SOC. A patient with separate events of the same PT will be counted only once in the frequency tables for that PT.

The following summaries will be provided:

- An overall summary of TEAEs, presenting the overall incidence (number and percentage) of subjects, by treatment group, for the following categories:
    - Subjects with at least one TEAE
    - Subjects with at least one severe TEAE
    - Subjects with any IP related TEAE
    - Subjects with any other-causes relationship TEAE
    - Subjects with at least one serious TEAE
    - Subjects with any TEAE resulting in death
    - Subjects with any TEAE with action taken marked as Surgical re-Intervention
    - Subjects with any TEAE with action taken marked as Surgical re-Intervention with same incision as in the Index Surgery.
- Tabulations of the number and percentage of subjects with at least one TEAE, as well as the number of events, summarized for each treatment group by System Organ Class (SOC) and by Preferred Term (PT) within SOC, will be provided for the following events:
    - All TEAEs
    - Serious TEAEs
    - Treatment-Related TEAEs
    - TEAEs resulting in death
    - TEAEs with action taken marked as Surgical re-Intervention with same incision as in the Index Surgery
    - TEAEs by maximal Severity (Mild, Moderate, Severe)
    - TEAEs by maximal relationship to Treatment (Not Related, , Related)

In addition, all AEs will be listed, regardless of whether they were treatment emergent. In addition, a listing of all SAEs will be provided. If there are any death, a by-patient listing of deaths will also be provided.

8.3. Physical Examination

Physical examinations (PE) will be performed at screening, Day 30 and Day 60 visits. As noted in the protocol, any abnormal finding with respect to baseline, assessed by the

Investigator as clinically significant, should be recorded as an Adverse Event in the relevant eCRF section.

In order to display any changes in body system findings over time, PE findings (Normal, Abnormal and not clinically significant, Abnormal and clinically significant, Missing) will be summarized using the appropriate descriptive statistics as specified in section 5.1 above, presenting:

- By-visit summary table;
- Shift table from baseline to each post-baseline scheduled visit.

In addition, all abnormal physical examination results will be listed.

8.4. Vital Signs

Vital signs(body temperature, systolic blood pressure, diastolic blood pressure and heart rate) are collected at screening and at Day 0 (before surgery, unless they were taken for screening occurring on the same day), Day 1, Day 5, Day 14, Day 30 and Day 60 (termination) visits. It should also be collected at unscheduled (UN) visits.
Each measurement will be assessed by the investigator as clinically significant (CS) or not clinically significant (not CS).
Vital signs measurements will be summarized using the appropriate descriptive statistics as specified in section 5.1 above, as follows:

- By-visit summaries will be presented on the raw and change from baseline values for each vital sign parameter (continuous).
- The number and percentage of subjects with not CS and CS values will be tabulated by visit, for each parameter. In addition, the number and percentage of subjects with not CS and CS values will be tabulated per parameter for any time during study time point, where a subject may be counted both as having CS value and as having not CS value, if experienced both events during the study.

Results obtained at unscheduled visit will not be included in by-visit summaries, but will be included in any time during study summaries, and data listings.

In addition, all clinically significant vital signs data will be listed.

8.5. Incisional Wound Healing

Incisional wound healing will be assessed by a blinded investigator, using visual examination (as a part of Surgical Site Assessment form and Surgical Site Assessment questionnaire form) and Modified Vancouver Scar Scale questionnaire. Assessment is scheduled at Day 1, Day 5, Day 14, Day 30 and Day 60 (termination) visit. Assessment will also be performed at UN visits.

Data will be summarized using the appropriate descriptive statistics as specified in section 5.1 above. The following will be provided:

- By-visit summaries for the following visual examination items:
    - Is the wound healing as expected: Yes, No, Not Applicable (categorical, based on Surgical Site Assessment form)
    - Was the wound suspected to be infected: Yes, No (categorical, based on Surgical Site Assessment form)
    - Final Assessor Decision (based on Surgical Site Assessment questionnaire form):
        - Superficial Surgical Site Infection: Yes, No (categorical)
        - Deep Surgical Site Infection: Yes, No (categorical)
- By-visit summaries for responses to the Modified Vancouver Scar Scale questionnaire (categorical).

In addition, all incisional wound healing data will be listed. Only scheduled visits will be summarized in by-visit analysis, but unscheduled visits will be presented in data listings.

8.6. Laboratory Tests

Laboratory hematology and chemistry Blood Tests are collected at each scheduled visit and are to be collected also at unscheduled visit, if performed. Urinalysis and coagulation tests are performed at Screening/pre surgery only. Hematology and chemistry tests are analyzed by a central laboratory. Any abnormal finding with respect to baseline, assessed by the Investigator as clinically significant, should be recorded as Adverse Events in the relevant eCRF form. List of laboratory parameters can be found in the study protocol (section 9.1.11 and 9.1.12).

The following will be provided for each laboratory parameter, and will be summarized using the appropriate descriptive statistics, as specified in section 5.1, and presented by treatment group:

- By-visit summaries on the raw and change from baseline values (continuous).
- Shift tables presenting the changes in laboratory values from baseline to each post-baseline scheduled visit, and overall (any time during study). For this purpose each measurement will be classified as below normal / within normal / above normal, according to the specified normal ranges for that parameter. Patient counts and percentages will be used to summarize the shifts. In shift analysis to any time during study, the worst outcome in both directions (below normal, above normal) will be considered. i.e. a subject may be counted both as a shift to below normal and as a shift to above normal, if experienced both events during the study.

Results obtained at unscheduled visit will not be included in by-visit summaries, but will be included in any time during treatment summaries, and data listings. For coagulation and urinalysis, baseline data will be described.

In addition, all laboratory data will be listed. A separate listing of abnormal tests will also be provided.

8.7. Other Safety Measures

8.7.1. Concomitant Medications

Per the protocol, all currently used concomitant medications, as well as all medications taken/prescribed to the subject throughout the study will be recorded in the electronic Case Report Form (eCRF).

All medications will be classified using the Anatomical Therapeutic Chemical (ATC) classification codes and preferred terms (PT) from the World Health Organization Drug Dictionary (WHO-DD, WHODrug-Global-B3). Medications with start date or stop date on or after the date of index surgery or ongoing at study completion will be considered concomitant medications. In case of partial or missing dates, the following imputation rules will be applied.

If the medication start date is incomplete, then it will be imputed as follows for the purpose of determining concomitant use:

- If the start date is completely missing, the start date will be equal to the date of index surgery. However, if the stop date is not missing and is before the date of index surgery, then the stop date will be used instead.
- If the start day is missing, the first day of the month will be used.
- If the start day and month are missing, then the first day of the first month (January) will be used.

If the medication stop date is incomplete, then it will be imputed as follows for the purpose of determining concomitant use:

- If the stop date is completely missing and the medication is not ongoing, the stop date will be equal to the date of completion/withdrawal.
- If the stop day is missing, the last day of the month will be used.
- If the stop day and month are missing, then the last day of the last month (December) will be used.

All concomitant medications data will be summarized by treatment group and overall using Safety population. The number and percentage of subjects with at least one medication term will be summarized by ATC Level 4 category and by PT within an ATC Level 4 for each treatment group and overall. The table will be sorted by overall descending frequency of ATC Level 4 and then, within an ATC Level 4, by overall descending frequency of PT.

In addition, summaries of antibiotics that were taken prior to and after the index surgery will be provided:

- Two separate tables will be produced (one for antibiotics taken prior to the index surgery and the other for antibiotics taken after the index surgery) in the same format as for all concomitant medications table, described above.
- An overall summary table of antibiotics, summarized by treatment group and overall, presenting the incidence (number and percentage) of subjects with antibiotics taken prior to and after the index surgery, overall and by route of administration.

All concomitant medications data will be listed.

8.7.2. Surgery Characteristics

Surgery characteristics will be summarized using both ITT and Safety analysis population. The summaries will be provided by treatment group and overall, using the appropriate descriptive statistics as specified in section 5.1 above. The following will be provided:

- Preoperative IV Antibiotic Given Prior to Surgery: Yes, No (categorical)
- Surgery Performed: Yes, No (categorical)
- Type of Surgery: Right Hemicolectomy, Left Hemicolectomy, Transverse Colectomy, Sigmoid Colectomy, Subtotal Colectomy, Total Colectomy, Proctocolectomy, Low Anterior Resection, Rectum Resection, Other (categorical)
- Length of Surgical Index Incision (cm) (continuous)
- Length of Surgical Index Incision: >10 – ≤ 20 cm, > 20 cm (categorical)
- Anastomosis Performed: Yes, No (categorical)
- Type of Anastomosis Performed: Ileocolonic, Ileorectal, Ileoanal, Colocolonic, Colorectal, Coloanal, Other (categorical)
- Surgery Included Stoma: Yes, No (categorical)
- Any Complications Encountered During the Surgery: Yes, No (categorical).

All surgery characteristics data will be listed.

8.7.3. Measures of Treatment Compliance

D-PLEX treatment will be administered during a single operative session directly visualized by the Investigator and recorded in the subject's source documents. The number and percentage of subjects receiving each D-PLEX dose (2 or 3 vials) will be summarized.

In addition, during the study potential options with IP preparation (Yes/No) and/or IP application (Yes/No) will be documented. These outcomes will be summarized.

All study drug administration data will be listed. Problems with IP preparation r application will be listed within Surgery Characteristics (section 8.7.2).

8.7.4. Hospitalizations (During Study)

Hospitalizations during study will be summarized by treatment group using the descriptive statistics as specified in section 5.1 above.

The following will be provided regarding the Index hospitalization:

- The duration (in days), as recorded in the eCRF (continuous)
- Was the hospitalization length as expected (Yes/No)

The following will be provided regarding additional hospitalizations (during study):

- Any hospitalization (Yes/No)
- Any readmission due to SSI (Yes/No)
- Any hospitalization due to Adverse Event (Yes/No)
- Any elective hospitalization (Yes/No)
- Any hospitalization in ICU (Yes/No)
- Total hospitalization days (continuous, according to eCRF).

In addition, listing of Hospitalizations, including prior hospitalizations, will be provided.

9. PHARMACOKINETIC (PK) ANALYSES

The PK population will consist of all subjects who have been treated with D-PLEX, and have had PK sampling. All PK data will be listed. Details for PK endpoints and analyses will be provided prior to breaking the blind and prior to the database lock.

10. STATISTICAL SOFTWARE

All summary tables, figures, listings and statistical analyses will be generated using SAS® software, version 9.3 or later.

11. SUMMARY OF CHANGES FROM LAST SAP VERSION

Version 4 of the SAP incorporate the following additions:

Section	Other sections impacted	Topic	Change description and rationale
5.3		Multiple comparisons and multiplicity	Documenting the interim-analysis adjusted alpha level for the primary endpoint.
7.4		Subgroup analysis	Adding subgroup analysis by mechanical bowl preparation (Yes/No).
3.2.2.2		Secondary endpoints	Adding descriptive statistics summary

Version 3 of the SAP incorporate changes according to the recent protocol amendment (version 06), along with the inclusion of further details and clarifications without any major change.

The most significant changes made to the text in this document between this version and version 2 are summarized in the table below. For changes made following protocol amendment, please refer to the detailed justification therein:

Section	Other sections impacted	Topic	Change description and rationale

3.2.1	3.3; 4.5; 7.2.1	Primary efficacy endpoint; Handling of re-intervention at the target site	In accordance with protocol amendment.
3.2.1;	5.4.1; 5.5;	An analysis window for was valid non-missing Day-30 information was defined	In response to the COVID-19 pandemic, affecting visiting times, in accordance with the FDA Conduct of Clinical Trials of Medical Products During the COVID-19 Public Health Emergency Guidance from 2020.
3.2.2.2, 3.2.3	7.3.2; 8.7.4	The lists of Additional Secondary efficacy endpoints and safety endpoints was updated.	In alignment with the protocol
3.3.1		Sample Size calculation: Update to the minimal and maximal sample size	New target numbers to enroll were calculated based on the change (decrease) in the estimated baseline SSI rate, this step was undertaken in response to the COVID-19 pandemic, affecting visiting times, in accordance with the FDA Conduct of Clinical Trials of Medical Products During the COVID-19 Public Health Emergency Guidance from 2020.
3.3.2 Interim analysis;	3.5.2	Cancellation of the planned blinded SSR at 500 subjects; inclusion of comparative interim analysis at 750 subjects milestone for stopping early for efficacy or futility, or unblinded sample size reassessment.	An interim analysis allowing early stopping the study for efficacy or futility along with an option to upsize sample size the study based on informed and rigid statistical rules was undertaken in response to increasing total enrollment and its impact on the study duration (where timelines already delayed due to the pandemic), in accordance with the FDA Conduct of Clinical Trials of Medical Products During

			the COVID-19 Public Health Emergency Guidance from 2020.
4.2 Analysis populations		ITT population	Addressing unexpected mistakenly enrolled patients who were not to have abdominal surgery.
5.3 Multiple Comparisons and multiplicity;	3.3 7.2.1	This section was updated to detail and pre-specify the planned method to control type 1 error due to changes in interim analysis plan.	Following the newly added option for stopping early for efficacy or futility, or unblinded sample size reassessment.
5.4.2	7.3.1.2	For the key secondary efficacy endpoints, sensitivity analysis for missing data due to reasons other than deaths was detailed.	
6.4		The tabulation of corrected values (post-randomization) in the value of stratification factors was added.	
Prior Medications		This section was cancelled, as no summary tables are planned for prior medications.	
8 Safety analyses		Detailed description of study collected safety data and forms was added, along with their planned descriptive statistics.	

Version 2 of the SAP incorporates changes and edits to the previous version of the SAP (version 1), aiming to:

- insert greater detail and provide further clarifications in response to the FDA recommendations;
- align with the latest versions of the Clinical Study Protocol (version 04) and the Case Report Form.

The most significant changes made to the text in version 2 relative to version 1 are summarized in the table below.

Topic	Change Description and Rational
Second key secondary efficacy endpoint definition	In the definition of the second secondary efficacy endpoint (number (percentage) of subjects with at least one score of ASEPSIS > 20): "any time during the study" had been changed to "measured within 30 days post abdominal index surgery", as defined in the protocol
Handling withdrawals and missing data	To address FDA recommendation: Further clarification had been made to emphasize that, in addition to the primary efficacy analyses, in all sensitivity and supportive analyses of the primary efficacy endpoint deaths and re-interventions in the target incision will be addressed as treatment failures.
Analyses using Cochran–Mantel–Haenszel (CMH) test	To address FDA recommendation: The specific SAS option had been added to further detail on how the CMH risk difference estimate (with 95% CI) will be computed.
Primary analysis table, summarizing treatment failure events	To address FDA recommendation: Primary analysis table, summarizing treatment failure events, had been rearranged to present first all death cases (and possible cases where death occurred along with other types of treatment failure).
Sensitivity and supportive analyses	To address FDA recommendation: The following sensitivity analysis had been removed: "Rather than treating re-intervention at the target incision site as treatment failure, taking the SSI outcome according to the determination of the independent adjudication committee".
Surgery characteristics	To align with the CRF: Surgery characteristics section had been updated and further detailed to include the CRF information.

12. LIST OF TABLES, FIGURES AND LISTINGS

The list of summary tables, figures and listings will be provided in a separate document.

13. REFERENCES

1. Ratitch, B., Lipkovich, I., O'Kelly, M. 2013. "Combining Analysis Results from Multiply Imputed Categorical Data." Proceedings of the Pharmaceutical SAS Users Group Conference (PharmaSUG 2013), paper SP03. Cary, NC: SAS Institute Inc.
2. Rubin, D.B. 1987. Multiple Imputation for Nonresponse in Surveys. New York: John Wiley and Sons.