



D-PLEX 312 Clinical Trial Protocol

Legacy Document Number: CL-0028

Study Number:	Study D-PLEX 312
Full Title of Trial	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.
Short Title	Safety and Efficacy of D-PLEX in the Prevention of Post Abdominal Surgery Incisional Infection.
Version of Protocol	Version 08
EU CT Number	2023-509698-21
IND Number	131060
Sponsor	PolyPid Ltd, 18 HaSivim Street, Petach Tikva, Israel, 4959376 Tel: +972-74-719-5700
Sponsor Protocol Number	Study D-PLEX 312
Phase of Trial	Phase III
Site(s)	About [REDACTED] centers in the US, Europe and Israel
Sponsor Representative	[REDACTED], MD Medical Director PolyPid Ltd., 18 HaSivim Street, Petach Tikva, Israel, 4959376 Tel: [REDACTED] [REDACTED]

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INVESTIGATOR SIGNATURE

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Protocol Version:	08
EU CT Number:	2023-509698-21
IND Number:	131060

I have read this clinical trial protocol and appendices and I agree to adhere to the requirements. I will provide copies of this clinical trial protocol and all pertinent information to the trial personnel under my supervision. I will discuss this material with them and ensure they are fully informed regarding the Investigational Product and the conduct of the trial.

I will conduct the trial in accordance with the clinical trial protocol, ICH GCP guidelines, as well as local regulations. I also accept respective revisions to the clinical trial protocol approved by authorized personnel of the Sponsor and by regulatory authorities.

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Principal Investigator (print name)

Principal Investigator (signature)

*Date***CONFIDENTIAL**



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DOCUMENT HISTORY

Revision	Date reviewed/revised	Changes
Version 01	NA	First Issue
Version 02	19 Aug 2020	Changes in study design following FDA review of the protocol
Version 03	08 Nov 2021	Changes in study design following study procedures clarifications, EU regulatory authorities requests harmonized in this version.
Version 04	06 Mar 2022	Instruction for use was updated following FDA's feedback.
Version 05	09 Mar 2023	Study design was updated to enrich the study population with subjects undergoing abdominal surgery with incision length >20 cm, following FDA recommendation
Version 06	19 Oct 2023	Consolidated protocol for transition of the clinical study in line with Regulation (EU) No 536/2014 (CTR).
Version 07	22 Feb 2024	Updating the EU CT number in line with Regulation (EU) No 536/2014 (CTR)
Version 08		Incorporate the U.S. approved drafted version 6- open-label Safety extension part was added in case of early stop for efficacy to recruit additional subjects for safety analysis. Adjustment to SAP revisions.

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2 LIST OF ABBREVIATIONS

AE - Adverse Event

AESI –Adverse Event of Special Interest

ASEPSIS - Additional treatment, Serous discharge, Erythema, Purulent exudate, Separation of deep tissue, Isolation of bacteria, Stay duration as inpatient

ALT(SGPT) - Alanine Aminotransferase

AST(SGOT) - Aspartate Transaminase

AUC – Area Under Curve

BMI - Body Mass Index

BP – Blood Pressure

[REDACTED]

CDC - Center for Disease Control

CDMS - Clinical Data Management System

CKD - Chronic Kidney Disease

Cm - Centimetres

CRF - Case Report Form

eCRF - Electronic Case Report Form

CRP - C-Reactive Protein

CVA - Cerebro Vascular Accident

DMC - Data Monitoring Committee

DSSI - Deep Surgical Site Infection

DSUR – Development Safety Update Report

EA - Enterobacter Aerogenes

EC/IRB - Ethics Committee/Independent Review Board

ESRD - End Stage Renal Disease

EU - European Union

G - Gram

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GCP - Good Clinical Practice

GMP - Good Manufacturing Practice

HR – Heart Rate

IAP - Interim Analysis Plan

IB - Investigator’s Brochure

ICF - Informed Consent Form

ICH - International Council for Harmonisation of Technical Requirements for Pharmaceuticals For Human Use

IP - Investigational Product

IPAL - Investigational Product Accountability Log

ISO - International Organization for Standardization

ITT - Intention to Treat

IV - Intravenous

IWRS - Interactive Web Randomization System

LDH - Lactate Dehydrogenase

MG - Milligram

MIC - Minimal Inhibitory Concentration

mITT - Modified Intention to Treat

MOH - Ministry of Health

MRSA - Methicillin-Resistant Staphylococcus Aureus

NHSN - National Healthcare Safety Network

NIS - National Inpatient Sample

OR - Operation Room

PI - Principal Investigator

PK - Pharmacokinetics

PLEX - Polymer-Lipid Encapsulation Matrix

PP - Per Protocol

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PT - Preferred Term

QA - Quality Assurance

QC - Quality Control

RBC - Red Blood Cell

SAP - Statistical Analysis Plan

SAR - Serious Adverse Reaction

SAE - Serious Adverse Event

SoC - Standard of Care

SOP - Standard Operating Procedure

SSI - Surgical Site Infection

SSSI - Superficial Surgical Site Infection

SUSAR - Suspected Unexpected Serious Adverse Reaction

TEAE - Treatment Emergent Adverse Event

TIA - Transient Ischemic Attack

WBC - White Blood Cell

WOCBP - Women of Childbearing Potential

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3 TRIAL PERSONNEL

3.1 SPONSOR'S REPRESENTATIVES

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4 SUMMARY

Study Number:	Study D-PLEX-312
Study Title:	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.
Phase:	Phase III
Objectives:	The primary objectives are as follows: • To assess the anti-infective efficacy of D-PLEX administered concomitantly with the Standard of Care (SoC) over a period of 30 days post operation, by preventing surgical site infection (SSI), defined as superficial and/or deep infection, in the target incision, compared to the SoC treated control arm. • To assess the safety of D-PLEX administered concomitantly with the Standard of Care (SoC).
Number of Centers:	About █ sites in the US, EU and Israel
Subject Population:	Male and female, 18 years old and above at screening, undergoing an elective colorectal surgery, involving colon or rectal resection. From protocol amendment 5, only patients with incision length > 20 cm will be recruited to the study. The study will enrol participants who reflect the demographic for clinically relevant population with regards to: age, gender, race and ethnicity. This population reflects the US and Europe population undergoing abdominal colorectal surgeries.
Number of Subjects:	The target sample size for this study relates to number of subjects with incision length >20 cm and is set at a minimum of █ subjects to be enrolled and randomized into the study, with a maximum of █ subjects (with a 1:1 allocation ratio, the number of subjects allocated to each treatment arm will be █ at minimum and up to █ at maximum). The final number of subjects for the Primary Endpoint analysis (with incision length >20 cm) will be determined following an unblinded interim analysis for futility, efficacy and sample size re-estimation, when about █ subjects (with incision

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	length >20 cm) will complete their 30 days (1 month) follow-up visit and be evaluated for primary endpoint. At the time of protocol amendment 5 submission, the study already enrolled a total of [REDACTED] subjects with incision length ≥ 7 cm has already been enrolled and randomized into the study, [REDACTED] of them with incision length >20 cm and [REDACTED] of them with incision length ≥ 7 cm and ≤ 20 cm, respectively. Thus, the overall number of subjects in the study is estimated as follows: the target population size for incision >20 cm ([REDACTED] to [REDACTED] subjects) plus the [REDACTED] subjects with incision length ≥ 7 cm and ≤ 20 cm. In total, [REDACTED] to [REDACTED] subjects will be enrolled into the study. During the time of interim analysis conduct, additional subjects may be enrolled to the study. In case of early stopping based on findings of efficacy in the unblinded interim analysis with about [REDACTED] recruited subjects with incision length >20 cm, in order to reach a total [REDACTED] patients, additional complementary active open-label extension will be initiated to recruit subjects with incision length >20 cm for safety monitoring only. During this extension, subjects will be recruited to receive D-PLEX + SoC treatment in an open-label design. These subjects will be followed up until Day 60/Termination visit.
Test Product:	D-PLEX is a new formulation of extended release of Doxycycline (as Doxycycline hyclate). Each 5g D-PLEX vial contains 54.6mg Doxycycline free base (1.09%), which is equivalent to 63mg Doxycycline hyclate (1.26%). D-PLEX is supplied as a sterile powder to be reconstituted to paste in the operating room, using the instructions detailed in section 10.1.3.1. D-PLEX is intended for single administration. The non-active components of the extended-release antibiotic formulation are [REDACTED] All formulation components are biodegradable.
Indication:	D-PLEX is indicated for prevention of post abdominal surgery incisional infection.
Other Drugs Required for the Study:	NA
Standard of Care:	The SoC for prophylactic antibiotic treatment is based on

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	international guidelines³. It will be consistent and standardized for all sites in the clinical study and is composed of IV antibiotic prior to surgery, with or without oral antibiotic combined with mechanical bowel preparation (IV antibiotic only, IV antibiotic with mechanical bowel preparation or IV antibiotic with oral antibiotics combined with mechanical bowel preparation). Each site will use the same pre-defined SoC for all subjects during the study. IV antibiotic treatment will be composed of the 1st, 2nd or 3rd generation of Cephalosporin family plus Metronidazole given within 60 minutes prior to surgery and should be discontinued maximum 24-hour post index surgery. In case of allergy to the Cephalosporin, Penicillin or Metronidazole families, other IV antibiotic may be used. In cases where oral antibiotic (one antibiotic from Aminoglycosides family with or without erythromycin or metronidazole) with mechanical bowel preparation (laxative (polyethylene glycol, sodium phosphate, or a magnesium citrate–based regimen) and/or a high-volume enema) will be used as well, it will be given within 48 hrs prior to surgery. Subjects will be stratified by incisional length (7 cm - ≤10 cm, >10 - ≤20 cm, >20 cm), type of prophylactic Standard of Care (IV antibiotic only, IV antibiotic with mechanical bowel preparation or IV antibiotic with oral antibiotics combined with mechanical bowel preparation) and by region (US or Europe + Israel).
Dose Administration:	D-PLEX dose is individualized, pending length of the abdominal target incision, 1-3 vials (5g each, a total max of 15g) in a single application. For incisions > 20 cm, the dose is 3 vials (15 g of D-PLEX). Application will be done at the time of initial closure of the abdominal wall target incision. Following closure of the fascia, D-PLEX reconstituted paste will be applied on the fascia suture line, followed by soft tissues of the abdominal wall along the whole length of the surgical wound (including muscle, fat and dermis). D-PLEX will not be applied on top of the skin (suture line).

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Duration of Dosing:	D-PLEX is administered as a single application. The active ingredient (Doxycycline) is continuously released for approximately 30 days.
Study Design:	This is a phase III, prospective, multinational, multicenter, randomized, controlled, two-arm, double blind (participant and outcome assessor) study. The study population includes male and female, 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. At the time of protocol amendment 5 submission, the study already enrolled [REDACTED] subjects with abdominal incision ≥ 7 cm, [REDACTED] of them with incision >20 cm. Starting from protocol amendment 5, the study include only subjects with abdominal incision >20 cm, to enrich the study population with longer incisions, to further explore the benefits seen in D-PLEX 311 study among this population and as recommended by the FDA. 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 incisional length (7 cm - ≤ 10 cm, >10 - ≤ 20 cm, >20 cm), type of SoC prophylactic antibiotic regimens (prophylactic IV antibiotic only; prophylactic IV antibiotic with mechanical bowel preparation or prophylactic IV antibiotic with oral antibiotics combined with mechanical bowel preparation) and by region (US 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 OR staff will be trained to maintain in confidence the treatment assignment and not to disclose it to other staff members. In addition, all aspects of data management and clean-up will be done using blinded datasets.

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Subjects randomized to the investigational arm will be treated with D-PLEX during the surgery (index procedure), as an adjunct to the SoC (see below). D-PLEX will be applied during the closure of the abdominal target incision. D-PLEX will not be re-administered if any re-intervention occurs.

Subjects randomized to the control arm will receive only prophylactic standard of care (SoC).

For both arms the prophylactic SoC will be consistent and standardized for all sites in the clinical study and is composed of IV antibiotic immediately prior to surgery, with or without oral antibiotic combined with mechanical bowel preparation (IV antibiotic only, IV antibiotic with mechanical bowel preparation or IV antibiotic with oral antibiotics combined with mechanical bowel preparation). Prophylactic IV antibiotics (Cephalosporin family plus Metronidazole) should be discontinued maximum 24-hour post index surgery. Each site will use the same pre-defined SoC for all its subjects during the study.

Post-operative care (excluding prophylactic antibiotics) will be performed per site SoC. Post-operative resumption of activity is at the discretion of the Investigator, based on the subject's medical condition.

Doxycycline pharmacokinetic sampling will be collected in selected sites from randomized subjects in these sites. About █ series (█ subjects from the D-PLEX + SoC arm) of █ samples per set will be analyzed. Spontaneous Adverse Events (AEs) including death will be recorded throughout the study. Pregnancy will be reported using a specific form and will be followed up until resolution.

The planned clinical program will establish the efficacy and safety of D-PLEX by the reduction in the number of abdominal wall incisional infections observed during 30 days after surgical index procedure. All subjects will be followed until Day 60/Termination visit for safety.

Primary Efficacy Endpoint:

- Infection rate as measured by the proportion of subjects with at least one abdominal target incisional infection event, occurring within 30 days post abdominal surgery

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	and determined by a blinded and independent adjudication committee, in the study population with longer incision (>20 cm). [abdominal incisional infection is defined as Deep Incisional Surgical Site Infection (DSSI) and/or Superficial Incisional Surgical Site Infection (SSSI)]. All-cause mortality and re-interventions through the abdominal incision (target) within 30 days post index surgery will be analysed as treatment failure. Reintervention is defined as re-opening through the same target incision, used for the original index surgery, in the operation room (OR). An independent and blinded adjudication committee will review each case suspected of SSI and will adjudicate if meets the SSI endpoint criteria. <u>Secondary Efficacy Endpoints:</u> <u>Key endpoints:</u> 1. Infection rate as measured by the proportion of subjects with at least one abdominal target incisional infection event, occurring within 30 days post abdominal (index) surgery and determined by a blinded and independent adjudication committee, in the population of subjects with target incision length >20 cm. 2. Infection rate as measured by the proportion of subjects with at least one abdominal target incisional infection event, occurring within 30 days post abdominal surgery and determined by a blinded and independent adjudication committee, in the overall study population (incision ≥ 7 cm). All-cause mortality and re-interventions through the abdominal incision (target) within 30 days post index surgery will be analysed as treatment failure. 3. Number (percent) of subjects with at least 1 score of ASEPSIS > 20, within 30 days post abdominal (index) surgery (population of subjects with target incision length >20 cm). <u>Additional endpoints</u> (population of subjects with target incision length >20 cm):
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- Incidence of SSSI during 30 days post index surgery.
- Incidence of DSSI during 30 days post index surgery.
- All-cause mortality rate within 30 days post randomization.
- All-cause mortality rate within 60 days post randomization.
- Time to adjudicated SSI during 30 days post index surgery.
- Number (percent) of subjects re-admitted during 30 days post index surgery (for any reason) that have experienced adjudicated SSI during this re-admission.
- Number (percent) of subjects who experienced at least 1 surgical re-intervention due to adjudicated SSIs during 30 days post index surgery.

SSI related Additional Endpoints:

- Number (percent) of subjects with adjudicated SSI where at least one Doxycycline-resistant bacteria have grown in bacteriology tests.
- Number (percent) of Doxycycline-resistant bacteria out of all bacteria that were cultured during bacteriology test from subjects with adjudicated SSI.
- Number (percent) of subjects who experienced adjudicated SSI during 30 days post index surgery that were treated by IV antibiotic.
- Number of IV antibiotic treatment days, administered to subjects who experience adjudicated SSI during 30 days post index surgery.
- Average of subjects' cumulative ASEPSIS assessment score (AUC) for subjects with adjudicated SSI during 30 days.
- Number (percent) of subjects with at least 1 score of ASEPSIS > 20 in subject with adjudicated SSI within 30 days post abdominal (index) surgery.

Identification of an SSI will be based on CDC/NHSN Patient Safety Component Manual criteria (January 2023, chapter 9)

Safety Evaluation:

The following safety parameters will be evaluated in this trial:

- AEs, physical examinations & vital signs.
- Incisional wound healing (including Hernia) will be assessed by a blinded Investigator, using a visual examination (part of a Surgical Site Assessment

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	questionnaire) as well as Modified Vancouver Scar Scale wound assessment questionnaires. • Safety laboratory parameters: hematology, chemistry. It is expected that each subject will be in the study for approximately 60 days.
Inclusion Criteria:	Subjects who meet all the following criteria will be eligible to participate in the study: 1. Subjects undergoing an elective colorectal surgery involving resection, with or without a stoma formation, that includes at least 1 abdominal incision (target incision) that is >20 cm. 2. Subjects are preoperative hemodynamically stable. (BP≤180/110 and ≥90/60 mmHg, and HR≤120 and ≥ 60 bpm, and temperature ≤37.5°C and ≥35.5°C). 3. Male or non-pregnant female. 4. Female of child-bearing potential should have a negative pregnancy test (serum or urine dipstick) prior to index procedure. <i>Note: All female subjects of child-bearing potential must agree to use a highly effective method of contraception consistently and correctly for the duration of the study (see Section 8.6 – CONTRACEPTIVE METHODS).</i> 5. Subjects' age 18 years old and above at screening. 6. Subjects who sign the written Informed Consent Form. 7. Subjects who are willing and able to participate and meet all study requirements. 8. Survival expectancy of at least 60 days post randomization.
Exclusion Criteria:	Subjects who meet any of the following exclusion criteria are prohibited from participating in the study: 1. Subjects with suspected/diagnosed intestinal perforation, intra-abdominal abscess, or any emergency/urgent colorectal surgery with acute intestinal obstruction (e.g., toxic colitis, ileus/subileus, megacolon, diverticulitis, volvulus, etc.) 2. Subjects who underwent an intra-abdominal surgery within

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	the last 6 months prior to randomization. 3. Subjects with any preoperative active infection or who are receiving any antibiotic therapy in the past 1 week prior to randomization, excluding pre-operative prophylaxis or antibiotics for the treatment of the disease that is the indication for surgery. 4. Subjects undergoing concomitant major procedures in addition to the colorectal resection. Female sterilization surgeries (i.e. salpingo-oophorectomy etc.), involvement of a small bowel procedure or cholecystectomy may be allowed, pending an advanced consultation and approval from the Sponsor. 5. Subjects who received any anti-cancer treatment within the last 4 weeks of surgery. 6. Subjects who received abdomen and/or pelvis area radiotherapy for colorectal cancer within the last 4 weeks of the planned abdominal surgery. 7. Subjects who received oral or IV Doxycycline or Tetracycline family antibiotics during the past 4 weeks prior to randomization. 8. Subjects with known allergy to Doxycycline and/or to the tetracycline family of drugs or to the D-PLEX's excipients. 9. Subjects with known allergies to more than 3 substances (an allergy questionnaire will be completed during the screening process). 10. Subjects with history of severe allergic reaction to any substance, having required treatment with intravenous steroids/intramuscular epinephrine, or who in the opinion of the PI is at high risk of developing severe allergic reactions. 11. Subjects with End Stage Renal Disease (ESRD/ CKD stage 5). 12. Subjects with severe hepatic impairment. 13. Subjects with chronic urticaria. 14. Subjects diagnosed with CVA within the past 6 months prior to randomization.
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	15. Subjects who underwent any abdominal surgery and current planned index surgery involves re-opening the scar of a prior abdominal surgery performed within the last 3 years. 16. Any subject with an active malignancy or with malignancy that has not been in complete clinical remission and without maintenance chemo or immunotherapy for at least 3 years. Excluding: Subjects with potentially resectable non-metastatic colorectal cancer, that is the reason for the index surgery. Subjects who have had carcinoma in situ (or other cancer "in situ = Stage 0"), or squamous cell carcinoma of the skin, or basal cell carcinoma of the skin. Subjects with any additional non-violent tumor that does not require treatment 4 weeks prior to the surgery, and throughout the entire study duration. 17. Subjects with other concurrent severe and/or uncontrolled medical condition. 18. Psychiatric or any other disorder that compromises ability to provide informed consent for participation in this study. 19. Chronic alcoholic or drug abuse subjects. 20. Pregnant or breast-feeding women or women of child-bearing potential who refuse or are prohibited of using an effective contraceptive method of birth control throughout study participation, including the safety follow-up period. 21. Subjects who received any investigational drug within 30 days or 5 half-lives prior to randomization to the study (whichever is longer) and through the study. 22. Subjects participating in any other interventional study. 23. Subjects who in the opinion of Investigator, are not eligible to participate in the study and/or to comply with the protocol requirements (e.g. due to a cognitive or medical condition).
Sample Size:	The target sample size for this study relates to the number of subjects with incision length >20 cm and is set to a minimum of

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■ subjects to be enrolled and randomized into the study with a maximum of ■ subjects. The number of subjects allocated to each treatment group will be ■ at minimum and up to ■ at maximum.

The final number of subjects with incision length >20 cm that are required for the Primary Endpoint analysis will be determined following an unblinded interim analysis for futility, efficacy and sample size re-estimation, when about ■ subjects (with incision length >20 cm) will complete their 30 days (1 month) follow-up visit and evaluated for primary endpoint.

The updated sample size calculation was based on powering the study with respect to the primary efficacy endpoint, considering the primary analysis of subjects with target incision length >20 cm.

The maximal sample size was calculated using treatment effect of D-PLEX+SOC from the completed phase 3 clinical study results for the sub-population with incision >20 cm, and the minimal sample size was based on an optimistic case based on the phase 2 clinical data results. For both the maximal and minimal calculations the assumed failure event rates in the SOC alone were as follows: (1) the SSI rate in the SOC arm would be ■%, (2) the Failure rate due to Re-intervention in the index surgery (included as Failure event in the primary endpoint analysis) = ■% and (3) the 30-day treatment failure rate due to all-cause mortality rate = ■%. The overall treatment failure rate in the SOC alone arm will be: ■% (■). For the maximal size calculation, it was assumed that D-PLEX +SOC will have effect of ■% on this rate (halving infection rate) relative to SOC alone, (thus ■% infection rate in D-PLEX+SOC arm), whereas in the minimal sample size calculation the effect was taken as ■%, thus leading to ■% infection rate in this arm. With the further assumptions regarding D-PLEX+SOC of no effect on mortality and ■% effect on failure due to re-intervention, the assumed overall treatment failure rates for D-PLEX+SOC were ■% (■) for the minimal size and ■% (■) for the maximal size. With these assumptions, sample size calculation showed that a total sample of ■ provides 90% power to detect the assumed difference in treatment failure rate using chi square test, at a

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	two-sided $\alpha=0.04$ level of significance, under the minimal sample size assumption whereas the required total sample size under the maximal size assumption is [REDACTED]. In order to account for an anticipated 5% lost-to-follow-up rate, the minimal sample size for this study was set at of [REDACTED] subjects ([REDACTED] treatment arm) with a maximum of [REDACTED] subjects ([REDACTED] per arm). The conservative significance level (<5%) was taken only for the purpose of the sample size calculation. The final sample size of the study will be determined based on results from a pre-specified interim analysis to be conducted when about a total of [REDACTED] subjects (with incision >20 cm) have been evaluated for the primary endpoint, and can be either about [REDACTED] (if the study to be stopped due to Efficacy or Futility), or [REDACTED] (if the study is to continue to planned initial size) or increased up to [REDACTED] in total, in accordance with the pre-specified comparative unblinded sample size re-estimation. During the time of interim analysis conduct, additional subjects may be enrolled to the study. In case of early stop for efficacy in the unblinded interim analysis with about [REDACTED] recruited subjects with incision length >20 cm, and in order to reach a total [REDACTED] patients, additional complementary active open-label extension part will be initiated to recruit additional subjects with incision length >20 cm for safety monitoring only. During this extension, subjects will be recruited to receive D-PLEX + SoC treatment in an open-label design. These subjects will be followed up until Day 60/Termination visit.
Sample size re-estimation:	One comparative interim analysis for an early efficacy or futility stops, or sample size re-estimation will be conducted when about the first [REDACTED] enrolled subjects with incision>20 cm will be evaluated for the primary endpoint. The interim analysis will include a group-sequential design stopping rule for efficacy and futility, combined with an 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, continuation to planned final ([REDACTED] subjects), or continue with sample size increased up to a maximum of [REDACTED] subjects, only if the comparative result falls in a predefined 'Promising Zone'.

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Randomization:	Subjects will be randomized to either the investigational arm (SoC + D-PLEX) or to the control arm (SoC only) in a 1:1 ratio. Randomization will be stratified by incisional length (7 cm - ≤10 cm, >10 - ≤20 cm, >20 cm), type of SoC prophylactic antibiotic regiments (prophylactic IV antibiotic only, prophylactic IV antibiotic with mechanical bowel preparation or prophylactic IV antibiotic with oral antibiotics combined with mechanical bowel preparation) and by region (US or Europe + Israel).
Statistical Methods:	The following analysis populations will be defined for the study: All-randomized: The all-randomized set will include all subjects that has been randomized in the study, regardless of their incision length category (>20 cm or not). In this analysis set, treatment will be assigned based on the treatment to which subjects were randomized, regardless of which treatment they actually receive. Intention to Treat (ITT): The ITT analysis set will consist of all subjects with planned target abdominal incision length >20 cm, who have been randomized to receive either D-PLEX + SoC or SoC alone. In this analysis set, treatment will be assigned based on the treatment to which subjects were randomized, regardless of which treatment they actually receive. Modified Intent to Treat (mITT): The mITT analysis set is a subset of the ITT set and will consist of all subjects randomized and treated with D-PLEX + SoC or SoC alone, who underwent the index surgery with actual incision length >20 cm and have no eligibility criteria deviation. In this analysis set, treatment will be assigned based on the treatment to which subjects were randomized, regardless of which treatment they actually received. The exclusion from the mITT population will be determined in a blinded manner prior to database lock. Subjects who will die during the study will not be excluded from the mITT population. Per Protocol (PP): The PP analysis set is a subset of the mITT set and will consist of all subjects in the mITT set that have no major protocol deviations and were assessed for the primary endpoint (i.e. completed the Day 30 assessment, unless had prior to that adjudicated post-surgical SSI, or death or re-intervention in the index surgery).

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Safety: The safety analysis set will consist of all subjects who have been randomized and treated. In this population, treatment will be assigned based upon the treatment the subjects actually received, regardless of the treatment to which they were randomized, their incision length, if have been ultimately operated or incision site (abdominal or not). Whereas active-open label extension part of the study will be initiated to recruit additional subjects for treatment with D-PLEX, these additional subjects will be included to safety analysis set and will contribute to Safety analysis only.

The analysis of the primary and key secondary (1st and 3rd) efficacy endpoint will be performed on the ITT, mITT and PP populations, with the ITT population serving as the primary analysis set. The 2nd key secondary endpoint will be using the all-randomized analysis set. Analyses additional secondary efficacy endpoints will be performed on the populations as described in the Statistical Analysis Plan (SAP) for the study.

Analyses of safety will be performed on the safety population.

Analysis of Primary Efficacy Endpoint

The primary analysis will be based on a composite treatment failure (Yes/No) variable indicating if a subject had at least one of the following treatment-failure events: SSI, as determined by a blinded and independent adjudication committee, within 30 days post index surgery, or all-cause mortality by 30 days. Re-interventions in the target incision within 30 days post index surgery and indeterminate SSI outcome will be considered a treatment failure for the primary ITT analysis. The primary population for the primary analysis is the subjects with target incision length >20 cm, per the ITT set definition.

The number and proportion of subjects with treatment-failure will be tabulated per treatment group. A 95% confidence interval will be constructed for each proportion. A Cochran Mantel-Haenszel (CMH) test will compare the proportion between the two groups, using the study stratification factors used for randomization, 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 hypotheses will be tested

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	for a significant difference in the primary endpoint proportions between D-PLEX+SoC (P_{DPLEX}) and SoC (P_{SoC}) treatment: $H_0: P_{DPLEX} = P_{SoC}$ vs. $H_1: P_{DPLEX} \neq P_{SoC}$ The significance level to be used in the final analysis will be the IA-adjusted significance level (namely as calculated based on the actual # of subjects used in the IA) to maintain the overall study - wise two-sided 5%. In the case of small number of events (less than 5 events in any study arm), the Fisher exact test will be used. The number and percent of each of the failure types will be described by group. Analysis of Safety All AEs will be coded using the MedDRA coding dictionary. The number and proportion of subjects reporting at least one treatment emergent adverse event (TEAE) and the number and proportion of subjects reporting at least one TEAE by MedDRA system organ class and preferred term will be reported. This analysis will be repeated for serious AEs (SAEs), Adverse Event of Special Interest (AESI) and for treatment-related AEs. Additional summaries of TEAEs will be performed by severity and by relationship to treatment. Vital sign measurements and clinical laboratory parameters will be summarized via descriptive statistics (mean, standard deviation, median, minimum, maximum, and number of non-missing observations) for each post-treatment time point. Shift tables will be constructed for physical exams to display any changes in body system findings over time. Responses to the Modified Vancouver Scar Scale will be summarized with counts and proportions by time point. All other analyses of efficacy and safety will be conducted as described in the main body of this clinical study protocol.
Interim analyses:	One comparative interim analysis for an early efficacy or futility stops or unblinded sample size re-estimation will be conducted, when about a total of [REDACTED] subjects (with incision length >20 cm) will be 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

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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 (■) subjects with incision length >20 cm) or continue with sample size increased up to maximal size of ■ only if the comparative result falls in a predefined 'Promising Zone'.

Blinding to comparative interim results

The unblinded interim analysis to be performed by an independent DMC statistician who is otherwise not involved in the study, according to a pre-planned interim analysis plan, and comparative information will be kept confidential within the DMC until the study is unblinded. Moreover, should the Interim analysis result lead to study size continuation/increase, the interim information provided to the sponsor will concern sample size only.

Final analysis

Shall the study not be stopped early for efficacy at the interim analysis, all final planned analyses will be performed only after the last subject has completed the study and the database has been locked. Prior the interim analysis, it is planned to lock the primary endpoint efficacy data of those subjects included in the interim analysis (as per the Interim Analysis section above). Only the primary endpoint will be formally tested as part of the IA. In the case of a successful IA result, the positivity of study and formal significance testing of the primary endpoint are to be based on the significant interim analysis result for the primary endpoint, and should additional subjects be available in the final full database (i.e. enrolled during the interim analysis period), it will serve to formally assess the key secondary endpoints where the primary endpoint will only be described and presented along with 95% confidence interval. No formal statistical testing will be done on the primary endpoint after the successful result from the interim analysis.

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5 INTRODUCTION

5.1 BACKGROUND

5.1.1 Background Information on The Product

5.1.1.1 Product Name

D-PLEX (Doxycycline/Polymer-Lipid Encapsulation Matrix)

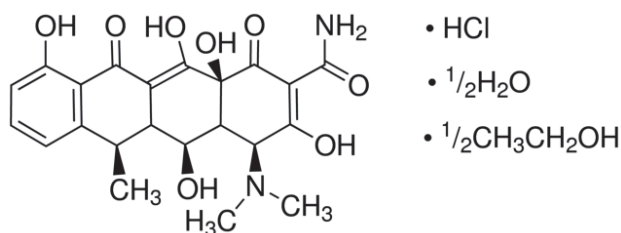
5g D-PLEX per vial contains 1.26% w/w Doxycycline hyclate equivalent to 1.09% (54.6mg) Doxycycline free base.

5.1.1.2 Drug Substance Chemical Name and Structure

Doxycycline Hyclate

The chemical name of Doxycycline hyclate is 2-Naphthacenecarboxamide, 4-(dimethylamino)-1,4,4a,5,5a, 6,11,12a-octahydro-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-, monohydrochloride, compound with ethanol (2:1), monohydrate, [4S-(4 α ,4a α ,5 α ,5a α ,6 α , 12a α)]- Doxycycline Hyclate chemical structure is shown in Figure 1 below.

Figure 1: Chemical Structure of Doxycycline Hyclate



Molecular Formula: $C_{22}H_{24}N_2O_8 \cdot HCl \cdot 0.5H_2O \cdot 0.5C_2H_5OH$

Molecular Weight: 512.9

5.1.1.3 Proposed Indication

D-PLEX is indicated for prevention of post abdominal surgery incisional infection.

5.1.1.4 Dosage Form, Route of Administration, and Dosing Regimen (Frequency and Duration)

Dosage form: D-PLEX is a biodegradable, extended-release formulation of Doxycycline (as doxycycline hyclate) supplied as a sterile powder for reconstitution and application as paste, and intended for a single use. D-PLEX is reconstituted with 2-5ml of sterile saline solution to form a paste.

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Each vial contains 5g of D-PLEX. The total percentage of Doxycycline in D-PLEX in the initial clinical formulation is 1.09% Doxycycline which is equivalent to 1.26% Doxycycline hyclate.

Reconstitution: Immediately before application, the entire content of D-PLEX vials is emptied into a sterile mixing bowl. The reconstitution is then performed as follows (according to required dose): one vial is reconstituted with 2mL sterile 0.9% saline solution, two vials are reconstituted with 3.5mL sterile 0.9% saline solution, three vials are reconstituted with 5mL sterile 0.9% saline solution to form a paste that is applied within the surgical site.

Route of administration: Following closure of the fascia, D-PLEX reconstituted paste will be applied on the fascia suture line, followed by soft tissues of the abdominal wall along the whole length of the surgical wound (including muscle, fat and dermis). Application is to be done at the time of initial closure of the abdominal wall incision. A thin layer of paste will be spread on the entire surface of each side of the abdominal incisional wall, except for the top of the skin (suture line), as an adjunct to the SoC treatment.

Dosing regimen: The product is to be administered on a single application prior to surgical wound closure following abdominal surgical procedures. The total dose applied at the surgical site will be determined based on the surgical target incision length. A maximum of three vials (15g) may be administered. Therefore, the total amount of Doxycycline free base for a single surgical procedure with D-PLEX can reach up to 163.8mg.

The individualized dose will be chosen in accordance with the following scheme:

Surgical target incision length (cm)	Number of vials to apply
7 - ≤10	1
>10 - ≤20	2
>20	3

5.1.1.5 D-PLEX Product

D-PLEX is a biodegradable formulation of [REDACTED], incorporating Doxycycline hyclate. D-PLEX is supplied in a glass vial (5g/vial) as a sterile powder to be reconstituted to paste and is intended for a single administration.

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5.1.1.6 Antibacterial Activity of the Drug Substance

Doxycycline is a well-known antibacterial drug substance that inhibits bacterial protein synthesis by binding to the 30S ribosomal subunit. Doxycycline has bacteriostatic activity against a broad range of Gram-positive and Gram-negative bacteria. Doxycycline is effective for eradication of most of the bacteria known to cause SSI.

5.1.2 Unmet Medical Need for Prevention of Post Abdominal Surgery Incisional Infection

Surgical site infections are one of the most frequent complications in open abdominal surgeries, and they represent a significant cause of mortality and morbidity. Approximately ten million (10,000,000) abdominal surgeries are performed annually in the US; of these, 7.1 million (7,100,000) are general (gastroenterological) surgeries which include 575,000 colorectal resection surgeries^{1,2}, 2.9 million (2,900,000) gynecological surgeries, and 608,000 urological surgeries.

The SSI rate ranges from approximately 5.0%-30.0% of abdominal soft tissue surgeries. SSIs occur in up to 4.0% of hysterectomies³, 30.0%⁴ of colorectal surgeries and approximately 6.8% in urological procedures⁵. Chemotherapy and other immuno-suppressive medications affect the function of the immune system and have the potential to increase the SSI rate⁶.

SSIs are classified as superficial incisional if limited to the skin and subcutaneous tissue, or deep incisional when involving the fascia and muscle. These infections are classified as organ space when involving an organ within the body cavity (i.e., below the fascia). Such organ space infections are usually manifested as abscess^{7,8}. Deep tissue and organ space surgical site infections are less frequently encountered than superficial infections, but are associated with greater morbidity/mortality, more readmission rates, longer hospital stay, long term antibiotic treatment and increased overall hospital-associated costs when compared to superficial surgical site infections. Although superficial wound infections are more reliably treated with systemic and/or topical antibiotics than deep wound infections, they can lead to clinically important complications such as non-healing wounds, systemic infections, surgical site revision surgeries, and, if hospitalization is pro-longed, an increased risk of developing co-infection with antimicrobial-resistant pathogens.

While many SSIs are uncomplicated, others may be severe and more challenging to manage, such as necrotizing deep soft tissue infections. The latter often requires extensive surgical debridement, multiple reoperations, and can be life-threatening⁷.

5.1.3 Justification for the Use of D-PLEX for Prevention of Post Abdominal Surgery Incisional Infection

D-PLEX represents a novel formulation of Doxycycline that improves the potential for the

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prevention of post abdominal surgery incisional infections over currently utilized preventive measures. Such measures include: (a) additionally applied mechanical measures; (b) administering prophylactic antibiotics prior to incision; (c) clipping rather than shaving the operative site; (d) maintaining normal body temperature and oxygen supplementation perioperatively, and achieving adequate glycemic control^{7,9-11}; (e) introduction of a sterile area in the operating room in order to restrict movement, and increased sterile airflow and air exchange in the proximity of the patient; and (f) wearing specialized gowning by surgeons ("space suits") to prevent airflow from the surgeon onto the patient. While these measures have reduced the rate of infection, they have not completely eliminated it^{8,12}. Therefore, the prevention of post abdominal surgery infection represents an unmet medical need. Part of the reason for the persistence of these types of infections despite the above procedures is because a local hematoma forms at the surgical site following surgery. This is a wet environment that encourages bacterial growth. In an attempt to reduce the hematoma, strict homeostasis and drainage are used. However, drains also constitute a risk for bacterial introduction. In addition, the blood supply and lymph fluid at the hematoma site are disrupted. Therefore, systemic antibiotics and immune defences have impaired ability to reach the affected areas, causing these areas to be more susceptible to proliferation of bacteria and subsequent development of infection. With low local prophylactic antibiotic concentrations, there is a risk of developing antibiotic resistant bacteria. Raising the systemic antibiotic dose to improve local exposures may cause systemic toxicity. In an attempt to over-come this problem, some surgeons apply antibiotics locally before closing of the incision. This antibiotic treatment is effective for very short durations of 1-2 hours and does not prevent development of bacterial infection¹³⁻¹⁶. In addition, the American Society of Health-System Pharmacists, Surgical Infection Society, Infectious Diseases Society of America, and Society for Healthcare Epidemiologists of America have all stated that lacking sufficient evidence, the use of currently available topical antibiotics cannot be recommended¹⁷. A product such as D-PLEX that provides sustained local high levels of Doxycycline has the potential of achieving a reduction in infection not achievable with present available products or procedures.

As indicated above, D-PLEX is a new extended-release formulation of Doxycycline for administration in the abdominal sites following surgery. D-PLEX releases Doxycycline immediately upon product administration and during approximately 30 days following administration. This is the period in which most of the laparotomy colon surgical site infection occur⁸. During this period, the expected local Doxycycline concentration from D-PLEX is well above the minimal inhibitory concentration (MIC) of sensitive organisms. By preventing growth of bacteria via high local concentration of Doxycycline at the surgical site during the four weeks post-administration, D-PLEX is intended to be effective for prevention of abdominal surgery site infections.

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5.2 NON-CLINICAL DATA

Non-clinical pharmacology studies, performed to demonstrate D-PLEX antimicrobial activity, include the following:

- Three studies in rabbit abdominal incision site infection model (each with a different clinically relevant bacteria: Methicillin-Resistant Staphylococcus Aureus (MRSA), Enterobacter aerogenes (EA), Enterococcus faecium (VRE)) showed that D-PLEX at an applied dose comparable to 5g administered to a 70kg adult on a mg/kg basis, demonstrates reduction of infection (as reflected in lesser clinical signs, no incidence of systemic temperature rise together with approximately 3-4 log bacterial load lower than groups that received only bacteria with or without placebo).
- In vitro antimicrobial studies demonstrated D-PLEX ability to reduce the load of six clinically relevant bacteria.
- These findings support the claim of antibacterial activity of D-PLEX in prevention of infections in soft tissue environment in human.

Non-clinical pharmacokinetic studies were performed to demonstrate Doxycycline local and systemic concentration after its administration.

- According to a local PK study in a rabbit abdominal surgical incision model, the Doxycycline concentrations in the abdominal incision area are maintained for considerable time above the MIC value of sensitive, clinically relevant bacterial species. A high concentration of Doxycycline is achieved up to ~5 hours after D-PLEX administration ($>30\mu\text{g/mL}$). This is followed by constant Doxycycline concentrations in the range of approximately $\sim 3\text{--}5\mu\text{g/mL}$ that is maintained for approximately 30 days. These levels are well above the MIC of the sensitive strain, Staphylococcus aureus (MIC of $1\mu\text{g/mL}$).
- D-PLEX's ability to release Doxycycline for a period of at least 29 days in vivo was shown in rats after subcutaneous administration of D-PLEX.

Non-clinical safety and toxicity studies, include the following:

- A safety and toxicity pivotal study in which D-PLEX was administered into an abdominal incision in miniature swine, shows that administration of D-PLEX comparable on a mg/kg basis to 20g D-PLEX dose administered to a 70-kg adult, demonstrates no macroscopic or microscopic adverse effect systemic effects and completed bio-degradation of D-PLEX by 6-months.
- The safety of D-PLEX administration in the abdominal wall incision (muscle, fat and subcutaneous) was investigated in rabbits and swine. D-PLEX had no local or systemic adverse effects and did not impair the healing of the incision nor increase the adhesions to the incision for about 30 days post-administration.
- Two safety studies performed to evaluate the peritoneum reaction to D-PLEX after 24 hours and 28 days from D-PLEX administration in case of intra-abdominal leakage in rat abdominal

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model showed that D-PLEX exposure (comparable on mg/kg basis to 15gr D-PLEX in a 70-kg adult) to abdominal cavity is safe.

Hence, D-PLEX can be considered safe and suitable for its intended use.

For detailed non-clinical studies summary of the D-PLEX, see D-PLEX Investigator's Brochure, #DV-0079.

5.3 CLINICAL DATA

D-PLEX 311 study - A 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 was performed in 62 sites in US, Europe and Israel. The study included a total of 977 subjects, that were scheduled for elective abdominal colorectal surgery involving resection and including at least 1 incision that is > 10 cm. The following were shown:

- Proportion of subjects, who experienced SSI, reintervention, or mortality within 30 days: 45 (9.3%) subjects on D-PLEX + SoC as compared to 59 (12.1%) subjects on SoC treatment arm (ITT population, N=974) didn't met the Primary Endpoint (p-value = 0.1520).
- In a prespecified subpopulation of the 423 subjects with incision length >20 cm the D-PLEX + SoC arm showed significantly fewer events of SSI, mortality or re-intervention when compared to the SoC arm, with 8.0% (4.7 - 12.5) in the D-PLEX + SoC and 17.5% (12.7 - 23.4) in the SoC arm (p=0.0032, ITT population, 54% effect of D-PLEX).
- In subpopulation of the subjects with incision length >20 cm the D-PLEX + SoC arm showed significantly fewer events of SSI when compared to the SoC arm, with 4.4% (2.0 - 8.2) in the D-PLEX + SoC and 9.7% (5.9 - 14.7) in the SoC arm (p=0.0410, ITT population, 55% effect).
- Twenty-six (26) subjects died during the first 30 days post-surgery, 15 subjects on SoC treatment arm and 11 subjects on D-PLEX + SoC treatment arm. According to blinded Investigator and Sponsor, these cases were not related to study drug. The number of subjects died within 30 days post-surgery in the subpopulation with incision length >20 cm was 10 subjects (4.7%) in the SoC treatment arm in compared to 6 subjects (2.8%) in the D-PLEX + SoC treatment arm.
- During the Follow-up period (60 days) 33 subjects died (26 of them died during the first 30 days post-surgery), 17 subjects on SoC treatment arm and 16 subjects on D-PLEX + SoC treatment arm. All death cases were assessed as unrelated by both blinded investigators and the sponsor.
- Total Number of Treatment Emerged Adverse Events (TEAEs) as reported by the Investigators is 1301 TEAEs from 381 (79.7%) subjects (N=478) on D-PLEX + SoC versus 1448 TEAEs reported from 398 (79.9%) subjects (N=498) on SoC treatment arm. There were no reported SUSARs during the study.
- PK data (from 17 subjects) demonstrated that the D-PLEX extended-release formulation releases Doxycycline constantly over a period of 30 days as well as that the systemic exposure of D-PLEX is lower than any dose of Doxycycline-based product administered

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orally; related to C_{max} compared to similar PK variables of the following Reference Listed Products, i.e., relative, non-head to head C_{max} comparisons to ACTICLATE, VIBRAMYCIN and DORYX (labelling information data) and a medical literature citation¹⁸.

- Twenty-four (24) subjects (N=488) on D-PLEX + SoC and 31 subjects (N=489) on SoC treatment arm discontinued the study. None of them discontinued the study due to AEs.
- Following the review of the study results, it can be concluded that D-PLEX administration in abdominal colorectal surgeries with incision >20 cm is established and safe for use.

D-PLEX 310 study - A Phase II, Prospective, Multicenter, Randomized, Controlled, Two-arm, Single Blind (Participant, Outcome assessor), Study to assess Safety and Efficacy of D-PLEX Administered Concomitantly with SoC, compared to a SoC treated control arm, in prevention of post abdominal surgery incisional infection was performed in 8 sites in Israel. The study included a total of 201 subjects, that were scheduled for elective abdominal colon surgery involving resection and including at least 1 incision that is ≥ 5 cm.

The following were shown:

- Proportion of subjects, who experienced SSI or mortality within 30 days: 10 subjects on D-PLEX + SoC as compared to 24 subjects on SoC treatment arm (ITT population, N=201) met the Primary Endpoint (p-value = 0.0086) with SSI prevention rate of 59%.
- In the mITT population (N=185) analysis, 9 subjects on D-PLEX + SoC as compared to 23 subjects on SoC treatment arm met the Primary Endpoint (p-value = 0.0066) with SSI prevention rate of 61%.
- In the PP population analysis (N=179) 7 subjects on D-PLEX + SoC as compared to 23 subjects on SoC treatment arm met the Primary Endpoint (p-value = 0.0024) with SSI prevention rate of 69%.
- PK data (in 20 subjects) demonstrated that the D-PLEX extended-release formulation releases Doxycycline constantly over a period of 30 days as well as that the systemic exposure of D-PLEX is lower than any dose of Doxycycline-based product administered orally; related to C_{max} compared to similar PK variables of the following Reference Listed Products, i.e., relative, non-head to head C_{max} comparisons to ACTICLATE, VIBRAMYCIN and DORYX (labelling information data) and a medical literature citation¹⁸.
- Three (3) subjects on SoC treatment arm died during the first 30 days post-surgery, while none on D-PLEX + SoC treatment arm. According to PIs, these cases were not related to study drug.
- During the Follow-up period (60 days) 5 subjects on SoC treatment arm died (3 of them died during the first 30 days post-surgery). According to PIs, these cases were not related to study drug.
- Total Number of Study Drug-Related Adverse Events (AEs) as assessed by the Investigators is 8 TEAEs reported from 8 subjects (N=99) on D-PLEX + SoC versus 18 TEAEs reported from 13 subjects (N=100) on SoC treatment arm.
- One (1) subject (N=101) on D-PLEX + SoC and 8 subjects (N=100) on SoC treatment arm

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discontinued the study. None of them discontinued the study due to AEs.

An additional Phase Ib/II clinical trial in the Sternal Surgical Site indication was conducted in Israel and included two parts: part 1, open label single arm, and part 2, randomized, controlled, single-blinded to assess the safety and efficacy of D-PLEX concomitantly with SoC vs. SoC alone, in the prevention of sternal wound infection post cardiac surgery for up to 24 weeks (6 months) following surgery. The study included a total of 81 subjects that were scheduled for open heart surgery, including but not limited to subjects with high risk factors for infection: 20 subjects in part 1 (all in the treatment arm) and 61 subjects in part 2 of the study (21 subjects in the SoC arm and 40 subjects in the treatment arm).

A total of 60 subjects were randomized to D-PLEX with SoC group. One subject withdrew consent before treatment.

In a final Clinical Study report for the study the following were shown:

- A single subject in the SoC group (4.8%) experienced a superficial surgical wound infection on Day 6 post-surgery.
- No subjects in the treatment group experienced any surgical wound infection (0%).
- No hospitalizations, re-admissions or surgical re-interventions due to SSI were reported in the study.
- No safety issues were indicated, and no AE/SAE related to D-PLEX has been reported. 18/21 subjects in the SoC group (85.7%) and 48/60 subjects (80%) in the D-PLEX with SoC group experienced AEs. One subject in the D-PLEX plus SoC group died due to cardiogenic shock on Day 6 post-surgery, not related to D-PLEX or the study procedure.
- PK data demonstrated that D-PLEX releases Doxycycline continuously over a period of 30 days as well as that the systemic exposure of D-PLEX is lower than any dose of Doxycycline-based product administered orally; related to C_{max} compared to ACTICLATE, VIBRAMYCIN and DORYX (labelling information data) and a medical literature citation¹⁸.

These results are highly clinically meaningful and statistically significant, and therefore support the robustness of D-PLEX in abdominal SSI prevention in subjects with incision >20 cm. In addition, in this study high safety profile of the D-PLEX was shown.

5.4 SAFETY PROFILE OF DOXYCYCLINE

Doxycycline has been on the market for over 5 decades and its side effects are well established. Information on adverse reactions of Doxycycline capsules/ tablets are provided in the IB.

Furthermore, given the total dose of Doxycycline present in D-PLEX (i.e., maximum 163.8mg), which is lower than the overall daily dose of systemically administered Doxycycline (200mg), and

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given it is being gradually released over a period of approximately 30 days, it is unlikely that clinically significant systemic levels will be present to give significant side effects. This is further supported by pharmacokinetic data derived from the completed phase II and phase III clinical studies. It shows detectable plasma levels of Doxycycline for well into 30 days following administration of the D-PLEX formulation.

5.5 POTENTIAL BENEFITS OF D-PLEX FOR PREVENTION OF POST ABDOMINAL SURGERY INCISIONAL INFECTION

- Prevention of SSI, which may occur within 30 days post-surgery.
- Significant improvement in prevention of SSI when used in surgical procedures that hold high risk of infection.
- Induces faster recovery and early return to normal activity as compared to SoC due to lower SSI rate.
- Reduction in the number of surgical re-interventions due to surgical site infections.
- Reduction in the number of hospitalizations and/or shortening hospitalization, thus providing financial savings and reducing hospital load.
- Used concomitantly with SoC.
- Ease of administration, no special training is required.
- Local administration of effective dose of Doxycycline during surgery (which results in local concentration above the MIC for Doxycycline sensitive bacteria), which cannot be achieved by systemic or current local solutions.
- Administration of low total dose of Doxycycline compared to the dose in oral administered Doxycycline capsules.
- Broad spectrum activity of Doxycycline (gram-negative and gram-positive).
- Low potential risk for development of bacterial resistance.
- D-PLEX excipients are biodegradable, widely used in the medicinal or medical device industry, and have an established history of safety and clinical use.
- Reduced risks of overall toxicity and adverse side effects due to minimization of systemic exposure.

5.6 POTENTIAL RISKS OF D-PLEX FOR PREVENTION OF POST ABDOMINAL SURGERY INCISIONAL INFECTIONS AND THEIR MITIGATIONS

Potential contamination of microorganisms and pyrogens by the aseptic product.

Mitigation:

- Clear and detailed instructions on how to store, administer and use.

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Potential for local toxicity by drug product, including irritation of the tissues.

Mitigations at the subject level:

- In case of serious local toxicity induced by D-PLEX, including irritation of the tissues, removal and washing out the product will be done by the surgeon.

Mitigations at the population level:

- Close follow up of subjects
- Safety Monitoring

Hypersensitivity to Doxycycline and to the Tetracycline family of antibiotics

Mitigation:

- Exclude susceptible subjects with known allergy to Doxycycline / Tetracycline family and/or three different allergens.
- In case of allergic reaction to Doxycycline, the administered D-PLEX can be removed by re-exploring the surgical target incision and washing out the D-PLEX.

Mitigations at the population level:

- Close follow up of subjects
- Safety Monitoring

Potential for development of bacteria resistant to Doxycycline and -treatment failure.

Mitigation:

- SoC concomitantly administered with D-PLEX provides systemic antibacterial coverage.
- In case of potential bacterial resistant to doxycycline, antibiotic treatment will be given per site medical practice.

Administration outside of the target surgery site.

Mitigations:

- Training of the surgeons participating in the clinical trials, as part of the study initiation and the protocol training.
- Clear instructions how to apply, detailed in section 10.1.3.

Inadequate antibacterial activity of the product

Mitigations:

- SoC concomitantly administered with D-PLEX provides systemic antibacterial coverage.

Photosensitivity reaction: exaggerated sun burn reaction.

Mitigations:

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- Close follow up of subjects

Potential Risks and Mitigations due to COVID-19 pandemic

Potential risks due to COVID-19 pandemic were evaluated according to “Guidance on the management of clinical trials during the Covid-19 (coronavirus pandemic)” risks and mitigations are presented below in the table below.

Risk	Mitigation
1. Subject may be infected with SARS-2-COV prior to enrollment to the study	Charlson Patient characterization: the updated Charlson co-morbidity index (CCI) that include question “Is this a COVID-19 patient?” is used (see Appendix 5).
2. Subject was diagnosed to be infected with SARS-2-COV and must undergo abdominal surgery	• Hospital has all facilities necessitate to operate COVID-19 patients as needed. • D-PLEX is a new formulation of extended release of Doxycycline to be administered locally as a single application during abdominal surgeries. Systemic exposure of Doxycycline from D-PLEX is lower than any dose of Doxycycline-based product administered orally. Therefore, no potential interaction is expected with any additional medications as needed in this situation.
3. Subject treatment can be interrupted due to arriving/visits issues	As D-PLEX is administered locally as a single application, there is no risk to interrupt the continuing treatment due to COVID-19 pandemic situation.
4. Study Follow-up visits can't be performed on site due to COVID-19 pandemic situation and safety of the subject can be compromised	• During the study, 4 of 7 visits are conducted during the primary hospitalization for the abdominal surgery. The additional 3 visits can be done by phone. • According to Table 1, “in case the subject is unable to attend a visit, information related to his wound assessment (as per Sponsor’s designated form) should be obtained in a phone conversation with him/his community physician/ a first degree relative”. • During remote visit, the patient will be asked to take photos of the incision area only, using their cellular phone per instructions provided as a separate manual to help the investigator and independent and blinded adjudication committee to perform wound assessment (see Section 9.1.18). • In case Central laboratory can't be used due to COVID-19 pandemic (home visits and/or connection disturbances), Local laboratories can be used to assess the safety and efficacy (bacteriology test) of the subject.

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5.7 SAFETY & EFFICACY SUMMARY

Based on the current data available from 3 clinical studies with D-PLEX, in which 586 subjects, who underwent abdominal surgery and 58 cardiac subjects, were treated with D-PLEX, no safety concern was observed. Overall, based on risk/benefit assessment, the expected benefits outweigh the expected risks associated with the use of D-PLEX.

Therefore, a clinical trial with D-PLEX in subjects undergoing abdominal surgery procedures, may be beneficial.

6 OBJECTIVES

- To assess the anti-infective efficacy of D-PLEX (administered concomitantly with SoC) over a period of 30 days post operation, by preventing 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.

7 STUDY ENDPOINTS

7.1 PRIMARY EFFICACY ENDPOINT

The following will be evaluated in this trial:

Infection rate as measured by the proportion of subjects with an SSI event, occurring within 30 days post abdominal (index) surgery and determined by a blinded and independent adjudication committee.

All-cause mortality and re-interventions through the abdominal incision (target) within 30 days post index surgery will be analysed as treatment failure.

SSI is defined as Deep Incisional Surgical Site Infection (DSSI) and/or Superficial Incisional Surgical Site Infection (SSSI).

Reintervention is defined as re-opening of the same target incision, used for the original index surgery, in the operation room (OR).

Superficial incisional SSI - Infection involves only skin and subcutaneous tissue of the incision and does not include diagnosis/treatment of cellulitis, a stich abscess alone or a localized stab wound or pin site infection.

Deep incisional SSI - Infection involves deep tissues, such as fascia and muscle layers; this also includes infection involving both superficial and deep incision sites.

An organ/space SSI (e.g. infection/abscess in the operated organ or peritoneal cavity) will not be accounted as an endpoint event.

Identification of an SSI will be based on CDC/NHSN Patient Safety Component Manual criteria (January 2023, chapter 9).

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The primary analysis of the primary endpoint will be based on subjects with target incision length >20 cm.

7.2 SECONDARY EFFICACY ENDPOINTS

7.2.1 Key endpoints:

1. Infection rate as measured by the proportion of subjects with at least one abdominal incisional infection event, occurring within 30 days post abdominal (index) surgery and determined by a blinded and independent adjudication committee.
[abdominal incisional infection is defined as Deep Incisional Surgical Site Infection (DSSI) and/or Superficial Incisional Surgical Site Infection (SSSI)].
2. The primary endpoint analyzed in the overall population with incision length ≥ 7 cm.
3. Number (percent) of subjects with at least 1 score of ASEPSIS > 20 within 30 days post abdominal (index) surgery.

7.2.2 Additional endpoints

- Incidence of SSSI during 30 days post index surgery.
- Incidence of DSSI during 30 days post index surgery.
- All-cause mortality rate within 30 days post randomization.
- All-cause mortality rate within 60 days post randomization.
- Time to adjudicated target incisional SSI during 30 days post index surgery.
- Number (percent) of subjects re-admitted during 30 days post index surgery (for any reason) that have experienced adjudicated SSI during this re-admission.
- Number (percent) of subjects who experienced at least 1 surgical re-intervention due to adjudicated SSI during 30 days post index surgery

7.2.2.1 SSI related Additional Endpoints:

- Number (percent) of subjects with adjudicated SSI where at least one Doxycycline-resistant bacteria have grown during the bacteriology test.
- Number (percent) of Doxycycline-resistant bacteria out of all bacteria that were grown during the bacteriological tests from subjects with adjudicated SSI.
- Number (percent) of subjects who experienced adjudicated SSI during 30 days post index surgery that was treated by IV antibiotic.
- Number of IV antibiotic treatment days, administered to subjects who experience adjudicated SSI during 30 days post index surgery.
- Average of subjects' cumulative ASEPSIS assessment score (AUC) for subjects with adjudicated SSI event during 30 days post -index surgery.
- Number (percent) of subjects with at least 1 score of ASEPSIS > 20 in subjects with

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adjudicated SSI within 30 days post abdominal (index) surgery.

7.3 SAFETY EVALUATION

The following safety parameters will be evaluated in this trial:

- AEs, physical examinations & vital signs.
- Incisional wound healing (including Hernia) will be assessed by a blinded Investigator, using a visual examination (part of a Surgical Site Assessment questionnaire) as well as *Modified Vancouver Scar Scale* wound assessment questionnaires.
- Safety laboratory parameters: hematology, chemistry.

8 TRIAL DESIGN

8.1 OVERALL DESIGN

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 target incision. The study will enrol participants who reflect the demographic for clinically relevant population with regards to age, gender, race and ethnicity. This population reflect the US and Europe population undergoing abdominal colorectal surgeries.

At the time of protocol amendment 5 submission, the study already enrolled ■■■ subjects with abdominal incision length ≥ 7 cm, ■■■ of them with incision >20 cm. Starting from protocol amendment 5, the study includes only subjects with abdominal incision >20 cm, that will enrich the study population with longer incision, following the D-PLEX 311 study results and as recommended by the FDA.

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 to either the investigational arm (SoC + D-PLEX) or to the control arm (SoC only) in a 1:1 ratio. Subjects will be stratified by incisional length (7 cm - ≤ 10 cm, >10 - ≤ 20 cm, >20 cm), by type of SoC prophylactic antibiotic regimens (prophylactic IV antibiotics only; prophylactic IV antibiotics with mechanical bowel preparation or prophylactic IV antibiotics with oral antibiotics combined with mechanical bowel preparation) and by region (US or Europe + Israel).

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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 OR staff will be trained to maintain in confidence the treatment assignment and not to disclose it to other staff members. In addition, all aspects of data management and clean-up should 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 per protocol.

Reintervention is defined as re-opening of the same target incision, used for the original index surgery, in the operation room (OR).

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 study. Each site will use the same pre-defined SoC for all its subjects during the study.

For subjects from both arms, preoperative IV antibiotics will be composed of 1st, 2nd or 3rd generation of Cephalosporin family plus Metronidazole given within 60 minutes prior to surgery. In case of allergy to the Cephalosporin, Penicillin families or Metronidazole, other IV antibiotic may be used. In cases where oral antibiotic (one antibiotic from Aminoglycosides family with or without erythromycin or metronidazole) with mechanical bowel preparation (laxative (polyethylene glycol, sodium phosphate, or a magnesium citrate-based regimen) and/or a high-volume enema) will be used as well, it will be given within 48 hrs prior to surgery.

Post-operative resumption of activity is at the discretion of the Investigator based on the subject's medical condition.

Doxycycline pharmacokinetic sampling will be collected in selected sites from randomized subjects in these sites. About █ series (█ subjects from the D-PLEX + SoC arm) of █ samples per set will be analyzed.

The occurrence of any Adverse Events (AE/SAE) including death will be recorded throughout the study.

The planned clinical study will evaluate the efficacy of D-PLEX in the prevention of SSIs over a period of 30 days for the primary outcome. All subjects will be followed for until Day 60/Termination visit for safety.

Option for an active open-label extension of the protocol

During the time of interim analysis conduct, additional subjects may be enrolled to the study. In the case that the planned unblinded interim analysis with █ recruited subjects with incision length >20 cm would lead to an early stop of the study for efficacy, in order to reach a total █

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subjects, additional complementary active open-label extension part will be initiated to recruit additional subjects with incision length >20 cm for safety monitoring only. During this extension, subjects will be recruited to receive D-PLEX + SoC treatment in an open-label design. These subjects will be followed up until Day 60/Termination visit, using the standard safety outcomes (adverse events, physical examinations & vital signs, safety laboratory parameters: hematology, chemistry, incisional wound healing [including hernia]). Subjects recruited to this extension part will be included in the safety analyses only.

8.2 DESCRIPTION & JUSTIFICATION OF THE DURATION OF TREATMENT, SUBJECT PARTICIPATION AND TRIAL FOLLOW-UP

The infection rate is measured within 30 days of index surgery, based on the literature that this is the time period in which most of the abdominal surgery surgical wound infections occur ^{3,14,15}.

The 60 days follow-up period was determined to allow a sufficient follow-up period to demonstrate the initial and sustainability safety endpoints of D-PLEX.

8.3 SELECTION OF SUBJECTS

At the time of protocol amendment 5 submission, the study already enrolled [REDACTED] subjects with abdominal incision ≥ 7 cm, [REDACTED] of them with incision >20 cm.

Starting from the protocol amendment 5, the study includes only subjects with abdominal incision >20 cm, that will enrich the study population with longer incision, following the D-PLEX 311 study results and as recommended by the FDA. The target sample size for this study relates to number of subjects with incision length >20 cm, and is set a minimum of [REDACTED] subjects to be enrolled and randomized into the study, with a maximum of [REDACTED] subjects (with a 1:1 allocation ratio, the number of subjects allocated to each treatment arm will be [REDACTED] at minimum and up to [REDACTED] at maximum).

The final number of subjects for the Primary Endpoint analysis (with incision length >20 cm) will be determined following an unblinded interim analysis for futility, efficacy and sample size re-estimation, when about [REDACTED] subjects (with incision length >20 cm) will complete their 30 days (1 month) follow-up visit and evaluated for primary endpoint.

See details in the section 14.3.8

8.4 INCLUSION CRITERIA

Deviations from any inclusion criteria are not allowed because such deviations can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety.

Therefore, adherence to the criteria as specified in the protocol is essential.

Subjects eligible for enrolment in the study must meet **all** the following criteria:

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1. Subjects undergoing an elective colorectal surgery involving resection, with or without a stoma formation, that includes at least 1 abdominal incision that is >20 cm (target incision).
2. Subjects are preoperative hemodynamically stable (BP≤180/110 and ≥90/60 mmHg, and HR≤120 and ≥60 bpm, and temperature ≤37.5°C and ≥35.5°C).
3. Male or non-pregnant female.
4. Female of child-bearing potential should have a negative pregnancy test (serum or urine dipstick) prior to index procedure. Note: *All female subjects of child-bearing potential must agree to use a highly effective method of contraception consistently and correctly for the duration of the study (see Section 8.6 – CONTRACEPTIVE METHODS).*
5. Subjects' age 18 years old and above at screening.
6. Subjects who sign the written Informed Consent Form.
7. Subjects who are willing and able to participate and meet all study requirements.
8. Survival expectancy of at least 60 days post randomization.

8.5 EXCLUSION CRITERIA

Deviations from any exclusion criteria are not allowed because such deviation can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety.

Therefore, adherence to the criteria as specified in the protocol is essential.

Subjects who meet **any** of the exclusion criteria are **prohibited** from participating in the study:

1. Subjects with suspected/diagnosed intestinal perforation, intra-abdominal abscess, or any emergency/urgent colorectal surgery with acute intestinal obstruction (e.g., toxic colitis, ileus/subileus, megacolon, diverticulitis, volvulus, etc).
2. Subjects who underwent an intra-abdominal surgery within the last 6 months prior to randomization.
3. Subjects with any preoperative infection or who are receiving any antibiotic therapy in the past one week prior to randomization, excluding pre-operative prophylaxis or antibiotics for the treatment of the disease that is the indication for surgery.
4. Subjects undergoing concomitant major procedures in addition to the colorectal resection. Female sterilization surgeries (i.e. salpingo-oophorectomy etc.), involvement of a small bowel procedure or cholecystectomy may be allowed, pending an advanced consultation and approval from the Sponsor.
5. Subject who received any anti-cancer treatment within the last 4 weeks of surgery
6. Subjects who received abdomen and/or pelvis area radiotherapy for colorectal cancer within the last 4 weeks of the planned abdominal surgery.

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7. Subjects who received oral or IV Doxycycline/Tetracycline family antibiotic during the past 4 weeks prior to randomization.
8. Subjects with known allergy to Doxycycline and/or to the tetracycline family of drugs or to the D-PLEX's excipients.
9. Subjects with known allergies to more than 3 substances (an allergy questionnaire will be completed during the screening process).
10. Subjects with history of severe allergic reaction to any substance, having required treatment with intravenous steroids/intramuscular epinephrine, or who in the opinion of the PI is at high risk of developing severe allergic reactions.
11. Subjects with End Stage Renal Disease (ESRD/CKD stage 5).
12. Subjects with severe hepatic impairment.
13. Subjects with chronic urticaria.
14. Subjects diagnosed with CVA within the past 6 months prior to randomization.
15. Subjects that undergone any abdominal surgery and current planned index surgery involves re-opening the scar of a prior abdominal surgery performed within the last 3 years.
16. Any subject with an active malignancy or with malignancy that has not been in complete clinical remission and without maintenance chemo or immunotherapy for at least 3 years.
Excluding:
Subjects with potentially resectable non-metastatic colorectal cancer that is the reason for the index surgery.
Subjects with carcinoma in situ (or other cancer "in situ = Stage 0"), or squamous cell carcinoma of the skin, or basal cell carcinoma of the skin.
Subjects with any additional non-violent tumor that does not require treatment 4 weeks prior to the surgery, and throughout the entire study duration.
17. Subjects with other concurrent severe and/or uncontrolled medical condition.
18. Psychiatric or any other disorder that compromises the ability to provide Informed Consent Form for participation in this study.
19. Chronic alcoholic or drug abuse subjects.
20. Pregnant or breast-feeding women or women of child-bearing potential who refuse or are prohibited of using an effective contraceptive method of birth control throughout study participation, including the safety follow-up period.
21. Subjects who received any investigational drug within 30 days or 5 half-lives prior to randomization to the study (whichever is longer) and through the study.
22. Subjects participating in any other interventional studies.

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23. Subjects who in the opinion of Investigator are not eligible to participate in the study and/or to comply with protocol requirements (e.g., due to a cognitive or medical condition).

8.6 CONTRACEPTIVE METHODS

Women of child bearing potential (WOCBP), will only be included in the study after confirmed menstrual period and a negative serum/dipstick pregnancy test. Women of childbearing potential (WOCBP) is defined as any woman or adolescent who has begun menstruation. A post-menopausal woman is defined as a woman who is over the age of 45 and has not had a menstrual period for at least 12 months.

All WOCBP must agree to use a highly effective contraception method from the following list for the duration of the study (until Visit 7 - Day 60):

- Combined (estrogen- and progestogen- containing) hormonal contraception associated with inhibition of ovulation¹ (oral, intravaginal, transdermal)
- Progestogen-only hormonal contraception associated with inhibition of ovulation¹ (oral, injectable, implantable²)
- Intrauterine device (IUD)²
- Intrauterine hormone-releasing system (IUS)²
- Bilateral tubal occlusion²
- Vasectomised partner ^{2,3}
- Sexual abstinence⁴

¹ Hormonal contraception may be susceptible to interaction with IMP, which may reduce the efficacy of the contraception method.

² Contraception methods that in the context of guidance are considered to have low user dependency.

³ Vasectomised partner is a highly effective contraception method provided that the partner is the sole sexual partner of the WOCBP trial participant and that the vasectomised partner has received medical assessment of the surgical success.

⁴ Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study intervention. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the subject.

Note: The following are not acceptable methods of contraception:

- Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea method (LAM).
- Male condom with cap, diaphragm, or sponge with spermicide.

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- Male and female condom should not be used together (due to risk of failure with friction).

8.7 RECRUITMENT

Subject recruitment will be conducted in general surgery departments at sites in US, Europe & Israel. Recruitment period is expected to take approximately 12 months.

9 STUDY PROCEDURES & SCHEDULE OF ASSESSMENTS

9.1 STUDY PROCEDURES

9.1.1 Informed Consent Procedure

An Informed Consent Form (ICF) will be prepared in accordance with the study protocol, ICH GCP and any applicable global and national regulations/requirements. The consent will include the information that data will be recorded, collected, processed and may be transferred to Competent Authorities. In accordance with the applicable global and national regulations, the data will not identify any subjects taking part in the study.

The final EC/IRB approved ICF and a copy of the approval must be submitted to the Sponsor prior to the initiation of subject enrolment at each investigational site.

Prior to any study-specific procedure or assessment, all subjects must document their consent, in writing, for study participation and authorization for use and disclosure of health information by signing the ICF. As part of the consent process, the subject will have the opportunity to ask questions and receive answers from the personnel conducting the study and will have ample time to consider and consult prior to providing his consent to participate in the study.

An original signed copy of the ICF will be retained at the investigational site and a copy of the signed consent form will be provided to the subject.

Procedures conducted as part of the subject's routine clinical management (e.g., imaging) and obtained prior to signing of the ICF may be used for screening or baseline purposes provided the procedure meets the protocol-defined criteria and has been performed within the timeframe specified in the protocol.

If during the study new information becomes available that can significantly affect the subject's future health and medical care, such information shall be provided to the respective affected subject in writing. If relevant, all affected subjects shall be asked to confirm their continuing informed consent in writing.

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9.1.2 Screen Failure

Subjects who sign the ICF, but do not meet the eligibility criteria and were not randomized will be considered as screen failures.

For such subjects, the reason for screen failure will be recorded in the screening log, the Case Report Form (CRF) and in the source documents.

9.1.3 Randomization / Enrolment

Upon site confirmation that the subject fulfils all inclusion criteria and none of the exclusion criteria are met, site staff will send signed "Patient eligibility confirmation form" to the Sponsor for approval (to e-mail [REDACTED]). After Sponsor's review and approval of the "Patient eligibility confirmation form" randomization could be made through an interactive web randomization system (IWRS) integrated with the eCRF.

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

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

Randomization will be stratified by incisional length (7 cm - ≤10 cm, >10 - ≤20 cm, >20 cm), by type of SoC prophylactic antibiotic regimens (prophylactic IV antibiotic only; prophylactic IV antibiotic with mechanical bowel preparation or prophylactic IV antibiotic with oral antibiotics combined with mechanical bowel preparation) and by region (US or Europe + Israel).

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

9.1.4 Blinding

This is a double-blind clinical trial. The sponsor, the subjects, outcomes 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 blinded to treatment assignment. In addition, all aspects of data management and clean-up will be done in blinded datasets.

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

When relevant, unblinded information (e.g., surgery report) will be reviewed by the unblinded site team and all reference to the allocated treatment arm will be removed, prior to filing of

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the relevant document to the patient study file.

Wound assessment, AE assessment and other study outcomes throughout the study follow-up visits will be done by a blinded Investigator, who will not be involved in the surgery.

An emergency card containing the Study Name, NIH number, Center Name/number, PI's name and contact details will be provided to the subjects. Subjects will be instructed to keep this card with them at all times and present it to medical staff in case of a medical urgency.

9.1.5 Emergency Unblinding

As a general rule, code breaks should occur only in exceptional circumstances when revealing of the actual treatment is absolutely essential for further management of the subject. For each study subject there is at least one Investigator (treating surgeon) that is unblinded to the study treatment. In occasion this person is unavailable and unblinding is deemed necessary and emergent, unblinding could be done by the unblinded Sponsor representative (24/7 support via phone call).

In general, the actual allocation will be not disclosed to the subject. In case it will be necessary for the subject's safety, the Investigator will disclose the actual treatment information to the said subject.

The Investigator is requested to maintain the blinding as much as possible. Investigators are encouraged to discuss unblinding in advance with a Sponsor's representative if he/she believes that unblinding is necessary. The actual treatment allocation must NOT be disclosed to the subject and/or other blinded study personnel including blinded site personnel, monitors, corporate Sponsors or project office staff.

In case an emergency unblinding is required due to an AE, AE reporting requirement must be followed (section 10.5).

The Investigator must report all code breaks (with reason) as they occur to the study monitor.

9.1.6 Assessment of the Infection Rate

Infection rate is measured by the proportion of subjects with an SSI event, determined by the blinded and independent adjudication committee, within 30 days post abdominal surgery. All-cause mortality and re-interventions through in the abdominal incision (target) within 30 days post index surgery, will be analysed as treatment failure.

Reintervention is defined as re-opening of the same target incision, used for the original index surgery, in the operation room (OR).

SSI is composed of Deep SSI and/or Superficial SSI.

Superficial incisional SSI -

Infection involves only skin and subcutaneous tissue of the

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incision and does not include diagnosis/treatment of cellulitis, a stitch abscess alone or a localized stab wound or pin site infection.

Deep incisional SSI -

Infection involves deep tissues, such as fascia and muscle layers; this also includes infection involving both superficial and deep incision sites.

An organ/space SSI (e.g. infection/abscess in the operated organ or peritoneal cavity) is excluded and will not be accounted as an endpoint event.

SSI will be identified by the predefined criteria based on CDC/NHSN Patient Safety Component Manual (January 2023, chapter 9).

Superficial Incisional SSI must meet the following criteria:

Date of event for infection occurs within 30 days after any NHSN operative procedure (where Day 1 = the procedure date)

AND

Involves only skin and subcutaneous tissue of the incision

AND

Patient has at least **one** of the following:

- a) Purulent drainage from the superficial incision
- b) Organisms identified from an aseptically-obtained specimen from the superficial incision or subcutaneous tissue by a culture or non-culture based microbiologic testing method which is performed for purposes of clinical diagnosis or treatment (for example, not Active Surveillance Culture/Testing (ASC/AST)).
- c) Superficial incision that is deliberately opened by a surgeon, physician or physician designee and culture or non-culture based testing of the superficial incision or subcutaneous tissue is not performed

AND

Patient has at least one of the following signs or symptoms: **(1)** localized pain or tenderness, **(2)** localized swelling, **(3)** erythema or **(4)** heat.

- d) Diagnosis of a Superficial Incisional SSI by the blinded investigator.

**The following do not qualify as criteria for meeting the NHSN definition of superficial incisional SSI:*

- *Diagnosis/treatment of cellulitis (redness/warmth/swelling), by itself, does not meet superficial incisional SSI criterion d.*
- *A stitch abscess alone (minimal inflammation and discharge confined to the points of suture*

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penetration).

- *A localized stab wound or pin site infection; depending on the depth, these infections might be considered either a skin (SKIN) or soft tissue (ST) infection.*

Deep Incisional SSI must meet the following criteria:

The date of event for infection occurs within 30 days after the operative procedure (where Day 1 = the procedure date)

AND

Involves deep soft tissues of the incision (e.g., fascia and muscle layers)

AND

Patient has at least **one** of the followings:

- a) Purulent drainage from the deep incision.
- b) A deep incision that, or is deliberately opened or aspirated by a physician or physician designee or spontaneously dehisces

AND

organism(s) identified from the deep soft tissues of the incision by a culture or non-culture based microbiologic testing method which is performed for purposes of clinical diagnosis or treatment (for example, not Active Surveillance Culture/Testing (ASC/AST)) or culture or non-culture based microbiologic testing method is not performed. A culture or non-culture based test from the deep soft tissues of the incision that has a negative finding does not meet this criterion.

AND

Patient has at least one of the following signs or symptoms: **(1)** fever (>38°C); **(2)** localized pain or **(3)** tenderness.

- c) An abscess or other evidence of infection involving the deep incision that is detected on gross anatomical or histopathologic exam, or imaging test.

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9.1.7 Management of Suspected SSI

In case of a suspected SSI, the following procedures should be carried out:

Action	Timeline
Collect Bacteriological Sample and ship into ICON central lab for testing	Once suspicion arises Immediately, BEFORE antibiotic treatment initiation
Collect Hematology/Chemistry Blood Samples for central lab	According to the investigator discretion
Perform a clinical assessment of surgical incision area by blinded Investigator	Immediately
Report Suspected Surgical Site Infection event through eCRF page	Within 24h of awareness
Photograph of the surgical wound in case of suspected infection	During incision assessment, BEFORE and after wound cleaning

All collected information and Investigator assessment of the suspected abdominal infection will be forwarded to the blinded “Endpoints Adjudication Committee” (EPAC).

EPAC will review all collected information and will confirm or exclude abdominal infection. EPAC will not intervene with the subject treatment.

In case of dispute between Investigator assessment of the infection and the committee’s assessment, the Endpoint Adjudication Committee’s assessment on primary endpoint evaluation only will overrule the Investigator assessment. At any case, the outcome of the Committee’s assessment will not be communicated to the investigator.

9.1.8 Safety Evaluation

The following safety parameters will be evaluated in this trial:

- AEs, physical examinations & vital signs.
- Incisional wound healing (including Hernia) will be assessed by visual examination (part of a Surgical Site Assessment questionnaire) by blinded Investigators. A *Modified Vancouver Scar Scale* questionnaire will be provided to Investigators to prompt meticulous evaluation of surgical wound for any suspicion of infection as well as evaluation of wound healing.
- Safety laboratory parameters: hematology, chemistry.

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9.1.9 Adverse Events

The Investigator and site staff are responsible for detecting, documenting, and reporting events that meet the definition of an AE. AEs will be collected from randomization, throughout the study, and until study termination at visit 7 (60 days after surgery).

AEs suspected to be related to the IP as well as SSI events and AESIs should be followed up until resolution or permanent outcome.

The study sponsor's medical representative will review and assess SAEs, AESIs and AEs suspected to be related to investigational medicinal product.

The Sponsor will adjudicate if a safety report meets the definition of SUSAR, based on the applicable regulations. Details on SUSAR reporting can be found in section 10.10 "REPORTINGS OF SUSARs".

In case of dispute between the Investigator and the Sponsor, the more severe assessment will prevail.

SSI will be handled as AEs and will be followed by the protocol's applicable sections for AE collection and reporting. Symptoms of SSI that do not meet an SSI diagnosis will be reported as AEs.

Pregnancy will be reported using a specific form and will be followed up until resolution.

The Data Monitoring Committee (DMC) will review all study safety data periodically until last subject last visit and provide its recommendation to the Sponsor if study may continue based on subjects' safety and any other ethical consideration as applicable.

Details on AE definition, assessment and reporting can be found in section 10.5 "RECORDING & REPORTING OF ADVERSE EVENTS & REACTIONS".

9.1.10 Laboratory Examinations

All protocol-required laboratory assessments must be conducted in accordance with the Central Laboratory Manual and the Protocol's Time and Events Schedule.

If any laboratory assessments are performed during the course of the trial and the result in a change in subject management (for example due to S/AE) the assessment data must be recorded in the subjects' eCRF.

9.1.11 Central Laboratory

Complete clinical chemistry and hematology samples will be collected and shipped to ICON Central Laboratory.

Laboratory requisition forms must be completed, and samples must be clearly labelled with the subject number, protocol number, site/ center number, and visit date. Details regarding the preparation and shipment of samples, and a list of reference ranges for all safety parameters will be provided by the central laboratory in the Central Laboratory manual.

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Clinical Chemistry Parameters

Albumin
 Alkaline Phosphatase
 Alanine Transaminase (ALT)
 Aspartate Aminotransferase (AST)
 Blood Urea Nitrogen (BUN)
 Calcium
 Chloride
 Creatinine
 C-Reactive Protein (CRP)
 Hemoglobin A1C (only at screening)
 Glucose
 Lactate Dehydrogenase (LDH)
 Phosphate
 Potassium
 Sodium
 Total Bilirubin
 Total Protein
 Creatinine Phosphokinase (CPK)

Hematology Parameters

Red Blood Cell (RBC) Count
 Hemoglobin (HGB)
 Hematocrit (HCT)
 Platelet Count
 White Blood Cell (WBC) Count
 WBC Differential (expressed as % and as absolute):
 Basophils
 Eosinophils
 Lymphocytes
 Monocytes
 Neutrophils

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9.1.12 Local Laboratory

Accreditations and reference range will be collected for all local laboratories and thereafter defined in the eCRF.

The following laboratory tests will be performed and analysed at the Screening visit and for baseline status only.

Coagulation Parameters – to be done at Screening visit
Prothrombin Time (PT)
Activated Partial Thromboplastin Time (aPTT)
International Normalized Ratio (INR)

Pregnancy test (β -HCG) by serum (for the central lab) or urine dipstick (for subject eligibility) at screening only, will be performed in female subjects of child-bearing potential only. In Portugal, pregnancy test will be performed also at Visit 6 (Day 30).

Local laboratory results can be used for Sponsor's approval of subject eligibility only in case where expedited approval is needed. In this case, local hematology and chemistry tests results (including CRP) will be sent to the Sponsor together with the "Patient eligibility confirmation form" to e-mail [REDACTED] and blood samples must be sent to the Central laboratory in parallel.

The result of HbA1c is not required for Sponsor's eligibility approval.

9.1.13 Bacteriological Testing

Should be Taken in Any Case of Suspected Infection Before Starting Antibiotic Treatment

Bacteriological tests (from the surgical site) will be performed in order to assess infection at the abdomen surgical target incision site.

Bacteriological tests will be taken in each visit when the index incision is suspected to be infected.

Prior to the collection of the swabs, the incisional site must be cleaned once with sterile 0.9% normal saline, and the sample must be taken directly from within the incision area that is suspected to be infected (superficial and deep tissue layers) excluding the incision edge or peri incision skin and not from drainage or any discharge.

When feasible, aseptically-obtained biopsy is preferred.

The test kit will be forwarded to ICON Central Laboratory where bacterial growth, identification and sensitivity to Doxycycline and other common practice antibiotics, will be assessed.

Results will be reported back to the referring Investigator.

Any abnormal finding, assessed by the Investigator as clinically significant, should be recorded

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as AE in the eCRF.

9.1.14 Physical Examination

The Investigator will perform a complete physical examination of the major body systems.

After randomization, any abnormal finding compared to baseline, should be recorded as an AE in the eCRF.

9.1.15 Vital Signs

Systolic/diastolic blood pressure, heart rate, & temperature measurements will be performed at each visit after 5 minutes of rest.

Weight and height will be measured, or the subject will be asked for this information at screening/admission.

All measurements will be recorded in the eCRF.

After randomization, any abnormal finding in the vital signs measurements stated above, assessed by the Investigator as clinically significant, should be recorded as an AE in the eCRF.

9.1.16 Medical History

Demographic data and a complete medical history of relevant past and present illnesses and surgeries/procedures as well as medications currently being taken to treat current illnesses will be recorded by the Investigator.

The medical history will include alcohol consumption, tobacco use, concurrent diseases, detailed allergy status (an allergy questionnaire will be completed) and assessment of possible infection. Based on the medical history, a Charlson co-morbidity index questionnaire will be completed for each potential subject.

Subjects with known allergy to Doxycycline and/or tetracycline family of drugs, or to D-PLEX's excipients should be excluded.

9.1.17 Allergy Questionnaire

An allergy questionnaire will be completed at screening as part of the subject's medical history.

9.1.18 Assessment of Surgical Site

A blinded Investigator/assessor will inspect the surgical site and assess the healing of the surgical wound. Special attention should be paid for signs of infection. The Investigator should follow the protocol guidelines for assessment of SSI (see section 9.1.6 above).

Surgical site assessment questionnaire, that includes potential symptoms of incision infection

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according to CDC criteria and ASEPSIS scoring, as well as modified Vancouver questionnaire¹ will be completed by the assessor.

To help the independent and blinded adjudication committee, in cases of a suspected infected wound, the assessor is asked to take a photographic picture of the incision area only (at each visit SSI is suspected/reported), using a cellular phone per instructions provided as a separate manual. The photo(s) should be taken prior to cleaning the incision and include the incision area with a sticker of the subject study number and date only and uploaded to a save location according to the instructions in the manual.

Every caution is to be made not to disclose any of the subjects' personal data or features such as tattoos.

Every effort must be made to assess the incisional surgical site at every visit. In case a subject is unwilling or unable to attend a scheduled visit, a phone conversation should be conducted with the subject, his first degree family relative or community physician and assessment of the wound per CDC should be done.

9.1.19 ASEPSIS: (Additional Treatment, Serous Discharge, Erythema, Purulent Exudate, Separation of Deep Tissue, Isolation of Bacteria, Stay Duration as Inpatient) Scoring Method

ASEPSIS is an acronym of wound assessment and treatment parameters, which provides numerically score during an inspection of the surgical site. The final score is being interpreted to severity of wound appearance and the clinical consequences of the infection.

Parameters are: serous exudate, erythema, purulent exudate, separation of deep tissue and also antibiotic therapy, drainage of pus under local/ general anaesthesia, isolation of pathogenic bacteria and stay as inpatient.

ASEPSIS questionnaire will be completed at each study visit, as part of the surgical site assessment.

9.1.20 Pharmacokinetics

Doxycycline pharmacokinetic sample will be collected in selected sites from randomized subjects in these sites. About 15 series (15 subjects from the D-PLEX + SoC arm) of 9 samples per set will be analyzed in total.

¹Modified Vancouver questionnaires will be completed starting from visit Day 14 onward, following wound closure

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Pharmacokinetic samples of 4ml blood will be collected at each PK time point, prepared, and frozen at -20°C or -80°C until shipment to the central laboratory. The concentration of doxycycline will be analyzed using a validated bioanalytical method.

Visit / Day	Time for sample collection
Screening (Visit 1)	Pre-application of D-PLEX (can be also done on Day 0 (Visit 2) before the Surgery)
Surgery - Day 0 (Visit 2)	Pre-application of D-PLEX (if not done during screening) AND 2-4 hours after D-PLEX Application
Day 1 (Visit 3)	24 hours after surgery
Day 2	48 hours after surgery
Day 3	72 hours after surgery
Day 5 (Visit 4)	5 days after surgery
Day 14 (Visit 5)	14 days after surgery
Month 1 (Visit 6)	30 days after surgery
Month 2 (Visit 7)	60 days after surgery

Samples must be clearly labelled with the subject number, protocol number, actual date and time sample was collected and site/center number.

Details for the preparation and shipment of samples are attached as appendix number 3.

9.2 STUDY VISITS & ASSESSMENTS

9.2.1 Visit 1: Screening Period (Day -21 – Day 0)

Subject will be screened for study eligibility at the clinic within 21 days prior to surgery (Day -21 – Day 0, when Day 0 is the day of surgery). The following screening assessments will be performed:

- Inform Consent process. Sign an ICF before any of the study related procedures are performed

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- Assign (from the eCRF) a sequential subject number. If a subject is withdrawn from the study or fails to undergo the treatment procedure, the subject number cannot be reassigned
- Record demographics (including age, gender, etc.)
- Record medical history and complete a Charlson co-morbidity index
- Complete an allergy questionnaire
- Record concomitant medications
- Measure height, weight and vital signs (body temperature, heart rate & blood pressure)
- Perform physical examination
- Collect blood for Hematology and Chemistry for Central Laboratory (and for local laboratory if an expedited Sponsor's approval is needed)*
- Send blood for Coagulation test to your local lab (tests done at the local lab which were performed as a routine procedure within 21 days prior to surgery and before ICF signature may be used for eligibility determination).
- Pre-application Doxycycline PK sampling at selected sites.
- Pregnancy test (if applicable) - could be done using serum either at the local or central lab or a urine dipstick**
- Record baseline conditions and planned procedures (any future planned elective procedure should be recorded so it will not be considered an S/AE)
- Eligibility determination according to inclusion and exclusion criteria

** Hematology and Chemistry tests should be performed not more than 7 days before surgery.*

***Pregnancy test should be performed not more than 1 day before surgery.*

9.2.2 Visit 2: Surgery – Index Procedure (Day 0)

Subjects will be enrolled into the study and will be treated with D-PLEX concomitantly with SoC or SoC alone.

The SoC for prophylactic antibiotic treatment, used in this study, is based on international guidelines^{3,19}. It will be consistently standardized for all sites in the clinical study and is composed of IV antibiotic immediately prior to surgery, with or without oral antibiotic combined with mechanical bowel preparation (IV antibiotic only, IV antibiotic with mechanical bowel preparation or IV antibiotic with oral antibiotics combined with mechanical bowel preparation). Each site will use the same pre-defined SoC for all its subjects during the study. The IV antibiotic treatment will be composed of the 1st, 2nd or 3rd generation of Cephalosporin family and Metronidazole given within 60 minutes before surgery and should be discontinued maximum 24-hour post index surgery. In case of allergy to the Cephalosporin, Penicillin families, or Metronidazole, other IV antibiotic may be used. In case where oral antibiotic (one antibiotic from Aminoglycosides family with or without erythromycin or metronidazole) with mechanical bowel preparation (laxative (polyethylene glycol, sodium phosphate, or a

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magnesium citrate–based regimen) and/or a high-volume enema) will be used as well, it will be given within 48 hrs prior surgery.

The following will be performed during visit 2:

- Confirmation of eligibility
- Randomization via the eCRF system (For surgery planned for early morning time, randomization via the eCRF could be done a day in advance).
- Vital signs before surgery, unless they were taken for screening occurring on the day of surgery
- Pre-application Doxycycline PK sampling (selected sites, if not done during screening)
- Abdominal Surgery
- D-PLEX administration (only for subjects randomized to D-PLEX treatment arm) at the stage of closure of the abdominal wall surgical incision
- Post-application Doxycycline PK sampling (at 2h - 4h) – selected sites
- AEs assessment and recording
- Concomitant Medication Review

9.2.3 Visits 3 – 6: Follow-Up Visits (Days 1, 5, 14 & 30 Post-Surgery)

Post-operative care will be performed per SoC. Post-operative resumption of activity is at the discretion of the Investigator based on the subject medical condition.

The subsequent follow-up visits (visits 3-5) will take place at Day 1, Day 5 and Day 14 post index surgery. These visits are usually performed after this type of surgery.

The additional follow-up visit (visit 6) will take place 30 days post index surgery and is required by the study, i.e., in addition to the routine visits usually performed after this kind of surgery.

During all the above-mentioned visits, the following procedures will be performed:

- Vital signs
- Assessment of surgical site, including visual examination:
 - Surgical Site Assessment questionnaires (on Day 1 - only if wound is not dressed).
 - Modified Vancouver questionnaire will be completed following wound closure from Day 14 onwards.
- Hematology and Chemistry for Central Laboratory
- Post-application Doxycycline PK sampling – selected sites
- AEs assessment and recording
- Concomitant Medication Review
- Physical examination at visit 6 (Day 30 only)
- Bacteriology cultures will be taken in each visit when the index incision suspected to be infected.

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- Perform pregnancy test (local serum test or urine dipstick) - on day 30 (for Portugal only).

9.2.4 Unscheduled Visit(s)

An unscheduled visit may be performed at any time during the study at the subject's request or as deemed necessary by the Investigator. Each study procedure performed to the subject during the unscheduled visit is to be updated in the eCRF. These procedures may be as following (done as one procedure or together with others):

- The date and reason for the unscheduled visit will be recorded.
- Assessment of surgical site,
- Vital signs,
- AEs assessment and recording
- Concomitant medications
- In addition, any procedures and evaluations may be performed as deemed necessary by the Investigator for the subject's safety.

This visit should be recorded in the eCRF, including all related procedure.

9.2.5 Visit 7 - End of Study (60 Days Post-Surgery)

- Vital signs
- Assessment of surgical site
- Hematology and Chemistry for Central Laboratory
- Post-application Doxycycline PK sampling – selected sites
- AEs assessment and recording
- Concomitant Medication Review
- Physical examination
- Bacteriology cultures (if required)

9.2.6 Study Assessments Summary

Table 1 details all planned study visits and assessments.

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TABLE 1: STUDY 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) ⁴	Visit 6 Day 30 (+7 days) ⁴	Visit 7 Termination Day 60 (± 7 days) ⁴
Informed Consent	X						
Medical History, Charlson co-morbidity index & Allergy questionnaire completion	X						
General Eligibility Criteria	X	X ¹					
Physical Exam	X					X	X
Vital Signs (blood pressure, HR, body temperature)	X	X	X	X	X	X	X
Weight & Height	X						
Pregnancy Test ⁷	X					X ⁷	
HbA1C and Coagulation Tests ³	X						
Assessment of Surgical Site (including SSA questionnaire) ⁵			X	X	X ⁴	X ⁴	X ⁴
Modified Vancouver Scar Scale					X	X	X
Blood Tests (hematology, chemistry ³)	X ²		X	X	X	X	X
Doxycycline PK Sampling ⁸	X	X	X	X	X	X	X
Bacteriology test and photographic picture ⁶			X ⁶	X ⁶	X ⁶	X ⁶	X ⁶
Adverse Events		X	X	X	X ⁴	X ⁴	X ⁴
Concomitant Medications	X	X	X	X	X	X	X
D-PLEX Administration		X					

Notes:

1. Confirmation of Eligibility.
2. Results must be available for PolyPid's MM review prior to randomization
3. Blood **chemistry and hematology**: for whole list of tests please refer section 9.1.11. Blood tests should be performed not more than 7 days before surgery. **Coagulation** tests (PT, INR, PTT) and HbA1C test only at Screening.
4. 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 sent (blinded with subject study number only) to the Sponsor study manager.
5. Surgical site assessment will be performed only if wound is not dressing
6. Bacteriology test and photographic picture will be performed in case of suspected infection (in each visit)

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7. Pregnancy test (serum or urine dipstick) should be performed within 1 day before surgery. On Day 30 it should be performed in Portugal sites only.

8. Blood samples for PK will be collected from about 15 treated subjects at selected sites at the following time-points: pre-D-PLEX application, 2-4 hours, 24 hours, 48 hours, 72 hours, 5 days, 14 days, 30 days and 60 days

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9.3 DEFINITION OF END OF TRIAL

The end of the trial will be the 60-days final follow-up visit for the last enrolled subject.

9.4 DISCONTINUATION/WITHDRAWAL OF PARTICIPANTS & 'STOPPING RULES'

Every effort will be made to follow all subjects and collect their data until the end of the study. Subject's participation in a clinical trial is voluntary, and the subject has the right to withdraw consent at any time without penalty, loss of benefit or prejudice to further treatment.

Although a subject is not obliged to give his/her reason(s) for withdrawing prematurely from a trial, the investigator should make a reasonable effort to ascertain the reason(s), while fully respecting the subject's rights.

In the event a subject withdraws consent, every effort should be made to have the subject return for a final study follow-up assessment and the information identified for collection at the 60 days follow-up visit will be obtained to the extent possible. Withdrawn subjects will not be replaced (the justified sample size considers an estimated allowance for attrition).

The reason for early termination should be recorded in the subject's medical records and in the eCRF. If more than one reason is cited for withdrawal, study personnel should identify the most significant reason.

The Investigator may discontinue a subject from participation in the study if the Investigator feels that the subject can no longer fully comply with the requirements of the study or if any of the study procedures are deemed potentially harmful to the subject.

The Sponsor may suspend or prematurely terminate this study either in an individual investigational site, or the entire study. Reasons for suspending or early terminating the study by Sponsor should be justified, documented and reported as applicable.

EC/IRB or regulatory authority may suspend or prematurely terminate participation in the study at the investigational sites for which they are responsible.

If suspicion of an unacceptable risk to subjects arises during the study, or when so instructed by the EC/IRB or regulatory authorities, the Sponsor shall suspend the clinical trial while the risk is assessed. The Sponsor shall terminate the study if an unacceptable risk is confirmed.

Data that has already been collected on withdrawn subjects will be retained and used for analysis, but no new data will be collected after withdrawal.

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10 NAME & DESCRIPTION OF ALL DRUGS USED IN THE TRIAL

D-PLEX is the IP to be administered in this study. A comprehensive description is provided below. No additional drugs will be administered as part of the requirements of this clinical study.

10.1 STUDY TREATMENT D-PLEX

10.1.1 Product Description

D-PLEX is a new formulation of extended release of Doxycycline. The total percentage of Doxycycline hyclate in D-PLEX is 1.26%, which is equivalent to 1.09% Doxycycline free base. Doxycycline is being constantly released for approximately 30 days following administration. D-PLEX is biodegradable.

D-PLEX is indicated for prevention of post abdominal surgery incisional infection.

This product is a sterile powder and is intended for a single use. Each 10ml vial contains 5g of D-PLEX.

10.1.2 Product Composition

D-PLEX contains the following substances:

Substance	Description
Doxycycline hyclate	Antibiotic
[REDACTED]	Release system
[REDACTED]	

10.1.3 Product Handling (storage & preparation)

D-PLEX is supplied as sterile powder to be reconstituted to paste in the OR and is intended for a single administration.

D-PLEX cannot be injected!

Each 10-ml vial contains 5g of D-PLEX.

D-PLEX should be stored at 2-8°C.

The product can be kept at room temperature for up to 12h until use (before vial opening). Avoid exposure to extreme heat.

D-PLEX powder will be hydrated in a bowl, using standard aseptic techniques, with sterile saline, immediately before administration, following the instruction described here:

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10.1.3.1 Product Preparation - Step 1:

To be conducted by a circulating nurse / non-sterile person from the study team.

NOTE: This step should be performed separately for each vial until its content is poured into a sterile bowl (sections 4-10). The contents of all the vials intended to be administered should be poured into the same bowl.

D-PLEX preparation should initiate only upon surgeon confirmation, as following vial opening, preparation and application should be done immediately.

The individualized dose will be chosen in accordance with the following scheme:

Surgical target incision length (cm)	Number of vials to prepare and apply
7-≤10	1
>10 - ≤20	2
>20	3

- Open the D-PLEX package and take out the required number of vials. **Caution: The outer surface of the vials is not sterile. Only the content of the vial is sterile.**
- Make sure that the integrity of the package and the vial is not damaged. **If it is damaged - do not use it.**
- Put on a pair of new, sterile gloves.
- Using 70% alcohol wipe in each hand, and without direct contact between the vial and the gloved hands, thoroughly wipe the entire vial (including the cap).
- Change your gloves to a new, sterile pair.
- Wait until alcohol evaporates.
- Shake the vial well.
- Ensure a mixing bowl is ready
- Remove the vial's cap by flipping the cap up and turning it counter clockwise, according to the arrows stamped on the cap.
- Open the vial slowly and do so close to a sterile mixing bowl using standard aseptic techniques.
- Without touching the bowl with the vial, pour the entire contents of the vial (5g) into the sterile mixing bowl, making sure that only the vial's neck is found above the sterile mixing bowl.
- In case not the entire contents of the vial was poured out, or a contact was made between the vial and the bowl, discard both the bowl and the vial and return to Step 1.

If further vials are needed (please see table above), repeat steps 4-10 above for the second vial and repeat again for the third vial. **The content of all the vials intended to be administered should be poured into the same bowl.**

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10.1.3.2 Product Preparation - Step 2:

To be conducted by a sterile team nurse/surgeon.

13. Slowly hydrate the D-PLEX powder in the bowl with the following volume of sterile saline.
D-PLEX powder from **1 vial** should be hydrated with **2ml** of sterile saline.
D-PLEX powder from **2 vials** should be hydrated with **3.5ml** of sterile saline.
D-PLEX powder from **3 vials** should be hydrated with **5ml** of sterile saline.
14. **Do not over hydrate.**
15. Mix the D-PLEX powder, with sterile saline using a sterile spatula, for approximately 1 minute, reaching a uniform paste appearance.
16. In case over hydration occurred and the paste is too diluted to be applied, discard it and return to Step 1 for new preparation of the D-PLEX paste.

10.1.3.3 Product Preparation - Step 3:

This step should be done by the operating surgeon.

17. The paste is to be applied by the surgeon, after wearing a new pair of sterile gloves.
18. **Administer** the whole quantity of the D-PLEX reconstituted paste **immediately** after hydration and mixing. The paste should be spread on the entire surface of each side of the abdominal incisional wall, along the whole length of the surgical wound (including muscle, fat, and dermis).
Do not apply on top of the skin and above the suture line.
19. In case application was not done immediately and the paste is over diluted, discard the paste and return to Step 1 with new vial(s) (package).
20. Packages (all 3 vials and box) should be kept for inventory documentation purposes and returned to the unblinded study coordinator after each surgery.

10.1.4 Product Usage and Dosage

The product is to be administered on a single occasion prior to surgical wound closure following abdominal surgical procedures, as an adjunct to the SoC treatment. The total dose applied at the surgical site will be determined based on the surgical incision length. A maximum of three vials (15g) may be administered. Therefore, the total amount of Doxycycline free base for a single surgical procedure with D-PLEX can reach up to 163.8mg.

The dose will be chosen according to the following scheme:

Surgical incision length (cm)	Number of vials to apply
7 - ≤10	1
>10 - ≤20	2
>20	3

D-PLEX will be applied directly within the surgical site immediately after hydration and mixing,

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using the same spatula.

Following the closure of the fascia, D-PLEX reconstituted paste will be applied on the fascia suture line and on the soft tissues of the abdominal wall along the whole length of the surgical wound (including muscle, fat and dermis). Application is to be done at the time of initial closure of the abdominal wall incision, a thin layer of paste will be spread on the entire surface of each side of the abdominal incisional wall, except for the top of the skin (suture line), as an adjunct to the SoC treatment.

10.1.5 Contra-Indications

Please refer to the exclusion criteria (section 8.5).

10.1.6 Use in Specific Populations

Please refer to the inclusion criteria in D-PLEX (section 8.4).

10.1.7 Warnings and Precautions

10.1.7.1 Clinical Warning and Precautions

- Proper surgical procedures are the responsibility of the surgeon.
- Each certified surgeon must evaluate the appropriateness of the procedure used, based on his/her medical training.
- Use sterile technique and equipment only.

10.1.7.2 Product Related Warnings and Precautions

- Keep the vial in its original package.
- Do not use vials which were not properly stored.
- Do not use vials which were not stored at 2-8°C.
- Use D-PLEX before the expiration date specified on the package.
- Do not use damaged vial or damaged package.
- Never use D-PLEX vial that was previously used, not even on the same subject.
- Avoid extended exposure to extreme heat, or humidity.

10.1.8 Overdose: Guidance on the recognition and treatment of possible overdose and adverse drug reactions

Overdose of D-PLEX is defined as using more than 3 vials of D-PLEX per subject (i.e. more than 15 gr per subject) in a single application. It is not expected to occur during the study as no more than maximum of 3 vials will be dispensed per subject.

Overdose of D-PLEX will be recognized by the report of number of vials used as documented by the operation room (OR) team.

In case of an overdose or adverse drug reaction, treat symptomatically and institute supportive measures. Dialysis does not alter serum half-life and thus would not be of benefit in treating cases of overdosage. Per surgeon decision, the administered D-PLEX can be removed by re-exploring the surgical incision and washing out the D-PLEX.

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All overdoses should be recorded and notified to the Sponsor.

If an SAE is associated with the overdose, the PI should ensure the overdose is fully described in the SAE report form.

Information on adverse drug reactions of D-PLEX are provided in the IB.

10.1.9 Packaging

D-PLEX is aseptically packed in sterile, de-pyrogenated amber (brown) glass vial and closed with a sterile and de-pyrogenated rubber stopper and aluminium flip-off cap.

Please note that the outer surface of the vial is not sterile. Only the vial content is sterile.

10.2 CONCOMITANT MEDICATION

10.2.1 Medication(s)/ Treatment(s) Permitted

Antibiotics Treatment per SoC Allowed in this Protocol:

- Prophylactic IV antibiotics with or without oral antibiotic combined with mechanical bowel preparation as per protocol (IV antibiotic only, IV antibiotic with mechanical bowel preparation or IV antibiotic with oral antibiotics combined with mechanical bowel preparation).
- Additional antibiotics (excluding Doxycycline) **for the treatment** of AE.

10.2.2 Medication(s) Prohibited During the Trial

- Additional prophylactic antibiotics (including antimicrobial sutures).
- Prolonged systemic steroid therapy (more than 80 mg per day of prednisone or equivalent) or any other form of immunosuppressive therapy.
- Doxycycline administration during the entire trial period.
- Any additional investigational drug

10.3 PREPARATION & LABELLING OF INVESTIGATIONAL PRODUCT (IP)

The Investigational Product (IP) is manufactured aseptically at [REDACTED], at a Grade A (ISO 5) clean room. The IP will be provided to the investigational site by [REDACTED] via [REDACTED] Ltd. depot. upon EC/IRB approval of the protocol and the ICF, Investigator Agreement is signed and all key documents required by GCP and local regulation have been collected.

D-PLEX is provided as a sterile and primarily packaged in a de-pyrogenated inert glass amber vial (10 ml), and each product vial contains 5g of D-PLEX. The sterile vial is packaged in a carton box.

Preparation and labelling of the IP will be completed in accordance with the relevant GMP guidelines.

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Information on the Product Handling (storage & preparation) can be found in section 10.1.3 of this protocol.

10.4 DRUG ACCOUNTABILITY

The Investigator or designee (i.e., SC, pharmacist) is responsible for ensuring adequate accountability of all used and unused study drug. This includes acknowledgment of receipt of each shipment of study product (quantity and condition) and recoding of subject dispensing, returned or destroyed study product. Dispensing records will document quantities received from the Sponsor and quantities dispensed to subjects, including lot number, date dispensed, subject identifier number, and the name of the person dispensing the medication.

All drug supplies and associated documentation will be periodically reviewed and verified by the study monitor over the course of the study.

Investigators will be notified in writing of enrolment completion. All unused D-PLEX vials must be returned to the Sponsor when enrolment is complete according to Sponsor's instructions.

10.5 RECORDING & REPORTING OF ADVERSE EVENTS & REACTIONS

The Investigator or designated site staff will be responsible for detecting, documenting and reporting events that meet the definition of an AE or SAE.

10.6 DEFINITIONS

10.6.1 Adverse Event (AE)

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment.

NOTE: An AE can therefore be any unfavourable and unintended sign (including an abnormal clinically significant laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product.

Examples of events meeting the definition of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or grade of the condition.
- New conditions detected or diagnosed after study treatment administration even though it may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction of study medication with any other treatment provided to the subject.
- Signs, symptoms, or the clinical sequela of suspected overdose of either study treatment or a concomitant medication (overdose per se will not be reported as an AE).

"Lack of efficacy" or "failure of expected pharmacology action" per se will **not** be reported as an AE or SAE. However, the signs and symptoms and/or clinical sequelae

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resulting from lack of efficacy will be reported if they fulfil the definition of an AE or SAE.

Events that **do not** meet the definition of an AE include:

- Medical or surgery procedure (e.g. endoscopy, appendectomy); the condition that leads to the procedure should be recorded as an AE.
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.
- The disease/ disorder being studied, or expected progression, signs, or symptoms of the disease/ disorder being studied, unless more severe than expected for the subject's condition.

10.6.2 Adverse Drug Reaction (ADR)

Any untoward and unintended response in a subject to an investigational medicinal product which is related to any dose administered to that subject. The definition implies a reasonable possibility of a causal relationship between the event and the IP.

This includes medication errors, uses outside of protocol (including misuse and abuse of product)

10.6.3 Serious Adverse Event (SAE)

SAE is any untoward medical occurrence that, at any dose:

- Death or Results in death
- Is life-threatening

NOTE: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it was more severe.

- Requires hospitalization or prolongation of existing hospitalization.

NOTE: In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting.

Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. When in doubt as whether "hospitalization" occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an SAE.

- Results in disability/incapacity

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NOTE: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

- Is a congenital anomaly/birth defect.
- Other important medical events

NOTE: Medical or scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasia or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

10.6.4 Unexpected Serious Adverse Reaction

A serious adverse reaction, the nature, severity or outcome of which is not consistent with the reference safety information (i.e. Investigator Brochure (IB)) in effect at the time of its occurrence.

10.6.5 Adverse Event of Special Interest (AESI)

An adverse event of special interest (serious or non-serious) is one of scientific and medical concern specific to the sponsor's product or program, for which ongoing monitoring and rapid communication by the investigator to the sponsor can be appropriate. Such an event might warrant further investigation in order to characterize and understand it. Depending on the nature of the event, rapid communication by the trial sponsor to other parties (e.g., regulators) might also be warranted. In this study, the following term is considered AESI: "Incision site impaired healing".

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10.7 ASSESSMENT OF ADVERSE EVENT

AEs and SAEs will be reported in accordance.

10.7.1 Severity

Category	Definition
Mild	The Adverse Event does not interfere with the volunteer's daily routine and does not require intervention; it causes slight discomfort.
Moderate	The Adverse Event interferes with some aspects of the volunteer's routine, or requires intervention, but is not damaging to health; it causes moderate discomfort.
Severe	The Adverse Event results in alteration, discomfort or disability which is clearly damaging to health.

10.7.2 Causality

Investigator's and Sponsor's assessment of causality (relatedness of the event to the study IP) is a clinical decision based on all available information at the time of the completion of the AE update within the eCRF. Investigator may change the causality assessment upon receive of new information such as test results, medical history etc.

The following categories will be used to define the causality of the Adverse Event:

Category	Definition
Related	A causal relationship between the IP and the reported event cannot be ruled out.
Not related	There is no evidence of any causal relationship between the IP and the reported event.

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10.7.3 Outcome

Category	Definition
Ongoing	AE is ongoing (i.e. worsened or without improvement) at the time of initial report. Amend the outcome as appropriate when additional data is available.
Resolved with Sequelae	Subject has recovered, but some signs/symptoms persist. The sequelae should not be reported as separate event but should be described in the narrative section of the AE reporting page.
Resolved	Subject has recovered and all AE/SAE's signs and symptoms were resolved.
Death	SAEs which were the primary cause of death and/or were directly responsible for subject's death should be reported with a fatal outcome. Where an AE/SAE is ongoing at the time of death, and was not considered the cause of death, the outcome must be recorded as "Ongoing".

10.7.4 Expectedness

Category	Definition
Expected	An AE that is classed in nature as serious and which is consistent with the information about the IP listed in the Investigator Brochure or clearly defined in this protocol.
Unexpected	An AE that is classed in nature as serious and which is not consistent with the information about the IP listed in the Investigator Brochure.

The reference document to be used to assess expectedness against the IP is the D-PLEX Investigator's Brochure.

10.7.5 Seriousness

Seriousness as defined for an SAE in section 10.6.3.

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10.8 DOCUMENTATION AND REPORTING OF SAE, AESI AND SSI

All SAEs, AESIs and SSIs should be reported to the Sponsor within 24 hours of knowledge by a **blinded** investigator.

SAEs, AESIs and SSIs will be reported through the study eCRF. In case the eCRF is not available, the blinded investigator will complete the Sponsor's SAE form and the form will be emailed to the Sponsor to the following email: [REDACTED] within 24 hours of knowledge.

It is the PI's responsibility to ensure SAE, AESIs and SSI queries raised by the Sponsor are responded as soon as possible.

The information should be completed as fully as possible and contain:

1. AE Term - if diagnosis was provided, the SAE term should be the diagnosis, otherwise, symptoms should be reported as separated AE/SAEs. SSI will be reported in the eCRF SSI event page.
2. Onset date – in case of SAE, report the start date the event became SAE.
3. End date - Report the date the subject's medical condition resolved or stabilised, or the date of discharge from hospital. Should signs and symptoms continued following discharge, report them as an AE
4. Seriousness criteria (if applicable)
5. Severity (mild, moderate, severe)
6. Relatedness to the surgical procedure
7. Relatedness to the IP (Related/Not Related)
8. Action taken to resolve the SAE (Treatment, Other Action)
9. Outcome (Ongoing, Resolved with Sequelae, Resolved, Death)
10. Narrative - brief and comprehensive, including clinical symptoms and accompanied with a simple, brief description of the event, Lab data results, timelines and other important information.

10.9 TIME PERIOD AND FREQUENCY FOR REPORTING AEs

The Investigator and site staff are responsible for detecting, documenting, and reporting events that meet the definition of an AEs (including SAE, AESI and SSI) will be collected from the time of randomization throughout the study, and until study termination at visit 7 (60 days after surgery). In addition, any SAE assessed as related to study participation (e.g., protocol-mandated procedures, invasive tests etc.), will be recorded from the time subject consents to participate in the study. After the termination of the clinical trial, newly reported SAEs which are suspected to be related to the IP by the study PI, and therefore reported to the Sponsor will be collected, documented, and reported as required by local and global regulations. All AEs, SAEs, AESIs and SSIs will be reported to Sponsor as indicated in the table below:

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	Initial Reports		Follow-up Reports	
Type of Event	Time Frame from awareness	Documents	Time Frame from Knowledge of New Information	Documents
SAEs	24 hours	eCRF	24 hours	eCRF
AESI	24 hours	eCRF	24 hours	eCRF
AEs which were assessed by the PI as 'not related' to the IP	5 days	eCRF	5 days	eCRF
AEs which were assessed by the PI as 'related' to the IP	24 hours	eCRF	24 hours	eCRF
All Suspected and/or Confirmed SSI events	24 hours	eCRF	24 hours	eCRF

10.9.1 Procedures for Recording & Reporting Serious Adverse Events

All SAEs will be recorded in the hospital's subject notes/system, the eCRF, and the Sponsor's SAE log.

10.9.2 Notification of Deaths

All deaths will be reported to the Sponsor irrespective of whether the death is related to a disease progression, the IP, or an unrelated event. Deaths should be reported within 24 hours of knowledge to the Sponsor through the AE page in the eCRF.

10.10 REPORTING OF SUSARs (SUSPECTED UNEXPECTED SERIOUS ADVERSE REACTION)

The Sponsor will notify SUSARs as applicable per local and global regulations. As a general rule, the Sponsor will notify SUSARs that are fatal or life-threatening to the Ministry of Health/Regulatory Authority and to the CEC/IRBs as soon as possible but within a maximum of 7 days; and the relevant follow up information is subsequently communicated within an additional 8 days. All other SUSARs shall be reported to the Ministry of Health/Regulatory Authority concerned and the EC/IRB as soon as possible but within a maximum of 15 days of first knowledge by the sponsor. The sponsor will also report cases of SUSARs to the Eudravigilance Clinical Trial Module (EVCTM) and will inform investigators involved in the trial, per local and global regulations.

Investigator will forward SUSARs that occurred at their center to the local EC/IRB in a timely manner, per local regulation. SUSARs will be forwarded to EC/IRBs periodically (at least every 6 months),

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based on local regulations.

10.11 DEVELOPMENT OF SAFETY UPDATE REPORTS

The Sponsor will prepare a Development Safety Update Report (DSUR) on an annual basis and submit it to ECs/IRBs and the Ministry of Health/Regulatory Authority per local and global regulations. The DSUR will contain an annual review and evaluation of pertinent safety information collected during the reporting period related to the IP.

10.12 THE TYPE & DURATION OF THE FOLLOW-UP OF SUBJECTS AFTER AES

AEs not related to the study product should be followed up until resolution, permanent outcome or subject's study termination, whichever is earliest.

AEs suspected to be related to the IP as well as AESIs and SSIs should be followed up until resolution or permanent outcome.

For SAEs, AESIs and SSIs- in case relevant information is not available at the time when the first report becomes available, the Investigator should report the known information within 24 hours of first awareness. Once additional information is received, the Investigator should fill in the missing follow-up information in the eCRF within 24 hours of the awareness of the new available information.

It is the responsibility of Investigators to inform the local Ethics Committee of SAEs (whether IP related or not) as required by the local Ethics Committee procedure and regulation.

11 NOTIFICATION OF PROTOCOL DEVIATION

The Investigator is responsible for ensuring the study is conducted in accordance with the procedures and evaluations described in this protocol.

It is within the site's Sponsor to notify the regulatory authority in writing of any serious breach of (i) The conditions and principles of GCP in connection with that trial; or (ii) The protocol relating to that trial, as amended from time to time, within 7 days of becoming aware of that breach.

The Sponsor will be notified immediately of any case where the above definition applies during the trial.

12 DATA MANAGEMENT & QUALITY ASSURANCE

12.1 CONFIDENTIALITY

The eCRF will not bear the subject's name or other personal identifiable data. The subject's date of birth and trial unique identification number will be used for identification.

12.2 DATA COLLECTION TOOLS & SOURCE DOCUMENT IDENTIFICATION

Primary data collection based on source-documented hospital and/or clinic chart reviews will

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be performed clearly and accurately by site personnel trained on the clinical trial protocol and eCRF completion. The Sponsor or designee will perform clinical monitoring throughout the study.

It is the responsibility of the Investigator to ensure the accuracy, completeness, legibility, and timeliness of all data reported to the Sponsor in the eCRFs and in all required reports.

12.3 DATA HANDLING & ANALYSIS

The procedures for data review and query management are described in the Data Management Plan and the Edit Checks Specification Table. Data will be reviewed throughout the study, in a blinded manner, according to these documents.

Data will be collected using eCRFs that are specifically designed for this study. The data collected on the eCRFs will be captured in a clinical data management system (CDMS) that meets the technical requirements described in 21 CFR Part 11 and EU regulations. The CDMS will be fully validated to ensure that it meets the scientific, regulatory, and logistical requirements of the study before it is used to capture data from this study. Before using the CDMS, all users will receive training on the system and study-specific training. After they are trained, users will be provided with individual system access rights.

Data will be collected at the investigational center by appropriately designated and trained personnel, and eCRFs must be completed for each screened subject according to their source documents.

Subject identity should not be discernible from the data provided on the eCRF.

A comprehensive validation check program will verify the data and automatically generate discrepancies for resolution by the Investigator.

Data will be verified using the source data by the study monitor and reviewed for consistency by data management using both automated logical checks and manual review.

All data collected will be approved by the Investigator at the investigational center. This approval acknowledges the Investigator's review and acceptance of the data as being complete and accurate.

13 RECORD KEEPING & ARCHIVING

The Investigator shall arrange for the retention of all study records for at least 15 years after completion of the study.

The Investigator should ensure that the following records are maintained:

1. Subject files containing copies of completed Case Report Forms and supporting documentation and the signed ICFs.
2. Investigator files containing copies of the documents required for the initiation of the study (e.g. signed Investigator's Agreement, Curricula Vitae for the principal and all sub-

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Investigators, copy of the EC/IRB approval of the protocol and Informed Consent Form, copies of correspondence with the CRO/Sponsor etc.). In addition to these records required by regulations, the Sponsor requests that the Investigator keep a copy of the Financial Agreement between CRO/Sponsor and the Investigator.

3. Files containing copies of the Investigational Medicinal Product Accountability Log (IPAL) or an equivalent form approved by CRO/Sponsor, and the Investigator's Brochure.

No records will be destroyed without the written consent of the Sponsor. It is the responsibility of the Sponsor to inform the Investigator when these documents no longer need to be retained.

14 STATISTICAL ANALYSIS CONSIDERATIONS

This protocol describes the statistical analysis as it is foreseen at the time of planning the study. A fully detailed Statistical Analysis Plan (SAP) will be produced and finalized after finalizing the protocol and before breaking the blind of 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.

14.1 SAMPLE SIZE AND POWER

The updated sample size calculation was based on powering the study with respect to the primary efficacy endpoint, considering the primary analysis of subjects with target incision length >20 cm.

The target sample size for this study relates to number of subjects with incision length >20 cm and is set to a minimum of [REDACTED] to be enrolled and randomized into the study with a maximum of [REDACTED] subjects in total. The sample size calculation was based on powering the study with respect to the primary efficacy endpoint, considering the primary analysis of subjects with target incision length >20 cm.

The final number of subjects for the Primary Endpoint analysis (with incision length >20 cm) will be determined following an unblinded interim analysis for futility, efficacy and sample size re-estimation, when about [REDACTED] subjects (with incision length >20 cm) will complete their 30 days (1 month) follow-up visit and evaluated for primary endpoint.

The maximal sample size for the Primary Endpoint analysis (with incision length >20 cm) was calculated using treatment effect of D-PLEX+SOC from the completed phase 3 clinical study results, and the minimal sample size was based an optimistic case based on the phase 2 clinical data results. For both the maximal and minimal calculations assumed failure event rates in the SOC alone were as follows: (1) the SSI rate in the SOC arm would be 11%, (2) the Failure rate due to Re-intervention in the index surgery (included as Failure event in the primary endpoint analysis) = [REDACTED]% and (3) the 30-day treatment failure rate due to all-cause mortality rate = [REDACTED]%. The overall treatment failure rate in the SOC alone arm will be: [REDACTED]% ([REDACTED]). For the

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maximal size calculation, it was assumed that D-PLEX +SOC will have effect of 50% on this rate (halving infection rate) relative to SOC alone (thus █% infection rate in D-PLEX+SOC arm), whereas in the minimal sample size calculation the effect was taken as █% thus leading to █% infection rate in this arm. With the further assumptions regarding D-PLEX+SOC of no effect on mortality and █% effect on failure due to re-intervention, the assumed overall treatment failure rates for D-PLEX+SOC were █% (██████████) for the minimal size and █% (██████████) for the maximal size. With these assumptions, a sample of █ total subjects provide 90% power to detect the assumed difference in treatment failure rate using chi square test, at a two-sided $\alpha=0.04$ level of significance. In order to account for an anticipated 5% lost-to-follow-up rate, a minimum of █ subjects (█ treatment arm) with a maximum of █ subjects (█ per arm).

At the time of protocol amendment 5 submission, a total of █ subjects with incision length ≥ 7 cm has already been enrolled into the study and thus the overall number of subjects in the study is estimated as the target for incision >20 cm (██████████) plus the █ subjects with incision length ≥ 7 cm and ≤ 20 cm. In total, █ to █ subjects with incision length ≥ 7 cm are expected to be enrolled into the study.

To note, the significance level for the above calculation was taken conservatively to account for the planned interim analysis for efficacy (to be performed with a two-sided $\alpha=0.01$). Details on the exact significance level that will be used in the final analysis (should the study not be stopped for efficacy at the interim analysis) are provided in section 14.1.1).

14.1.1 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 stops, or sample size re-estimation will be conducted when the first █ enrolled subjects with incision >20 cm will complete their 1-month Follow-up and evaluated for the primary endpoint. The interim analysis will include a group-sequential design stopping rule for efficacy and 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 continuation to planned final (█ subjects) or continue with sample size increased up to a total of █ 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 kept confidential until the study finally unblinded. The pre-defined stopping rule and the sample size re-calculation will be detailed in an Interim analysis Plan to be finalized before the unblinding takes place, in order to minimize potential for operational bias.

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14.2 ANALYSIS POPULATIONS

The following analysis populations will be defined for the study:

14.2.1 All-Randomized Set

The all-randomized set will include all subjects that has been randomized to receive either D-PLEX plus SoC or SoC alone, regardless of their incision length category (>20 cm or not). In this analysis set, treatment will be assigned based on the treatment to which subjects were randomized, regardless of which treatment they actually received. In addition, this analysis set will include randomized subjects even if eventually their abdominal surgery was not performed (after already randomized). Subjects randomized by mistake for whom target incision was not abdominal will not be included.

14.2.2 Intention to Treat (ITT) Set

The ITT analysis set will consist of all subjects who have been randomized to receive either D-PLEX plus SoC or SoC alone with planned target abdominal incision length >20 cm. In this analysis set, treatment will be assigned based on the treatment to which subjects were randomized, regardless of which treatment they actually received. In addition, this analysis set will include subjects even if eventually their abdominal surgery was not performed (after already randomized). Subjects randomized by mistake for whom target incision was not abdominal will not be included.

14.2.3 Safety Analysis Set

The safety analysis set will consist of all subjects who have been randomized and treated with D-PLEX or SoC alone. In this population, treatment will be assigned based upon the treatment the subjects actually received, regardless of the treatment to which they were randomized, their incision length, if have been ultimately operated or incision site (abdominal or not). Whereas active-open label extension part of the study will be initiated to recruit additional subjects for treatment with D-PLEX (as noted in section 8.1), these additional patients will be included to safety analysis set and will contribute to Safety analysis only.

14.2.4 Modified Intention to Treat (mITT) Set

The mITT analysis set is a subset of the ITT set and will consist of all subjects randomized and treated with D-PLEX+SoC or SoC alone who underwent the abdominal (index) surgery with actual incision length >20 cm and have no eligibility criteria deviation. In this population, treatment will be assigned based upon the treatment to which subjects were randomized, regardless of the treatment they actually received. Exclusion from the mITT population will be determined in a blinded manner prior to database lock and will be based only on observations at baseline.

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14.2.5 Per Protocol (PP) Set

The PP analysis set is a subset of the mITT set and will consist of all subjects in the mITT who have no major protocol deviations and were assessed for the primary endpoint (i.e. completed the Day 30 assessment or otherwise had prior to that adjudicated post-surgical SSI, or death or re-intervention in the index surgery).

The analysis of the primary and the 1st and 3rd key secondary efficacy endpoints will be performed on the ITT, mITT and PP populations, with the ITT population serving as the primary analysis set. The 2nd key secondary endpoint will be analyzed using the All-randomized analysis set. Analyses of additional secondary efficacy endpoints will be performed on the populations as described in the Statistical Analysis Plan (SAP) for the study. Analyses of safety will be performed on the safety analysis set. Baseline subject characteristics and demographics will be summarized for the enrolled and ITT populations.

14.3 STATISTICAL AND ANALYTICAL PLANS

14.3.1 Analysis of Demographics and Baseline Subject Characteristics

Baseline and demographic characteristics will be summarized for all subjects in the all-randomized and ITT populations by treatment group and overall. Continuous variables will be displayed via summary statistics (number of non-missing observations, mean, median, standard deviation, 1st and 3rd quartiles, minimum, and maximum). Categorical variables will be summarized via counts and percentages.

14.3.2 Primary Endpoint Analysis

The primary objective of the study is to assess the anti-infective efficacy of D-PLEX+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, in the population of subjects with target incision length >20 cm.

The primary analysis will be based on a composite treatment-failure (Yes/No) variable, indicating if a subject had at least one treatment-failure event, defined as follows: SSI, occurring within 30 days post abdominal surgery (up to and including the 30-days follow-up visit) and determined by a blinded and independent adjudication committee, or death (from any reason) within 30-days from surgery. In addition, re-intervention at the target incision site within 30 days of the index operation (regardless of the panel's determination) will be considered a treatment failure for the primary ITT analysis. To note, re-intervention at a different access point than the index wound outcome will not be assessed for SSI and the outcome for the index incision will be determined based on the adjudication panel. In the rare case of indeterminate SSI outcome (i.e. missing outcome due to other reasons than death), it will also be counted as treatment failures for the primary analysis.

The primary population for the primary analysis is the subjects with target incision >20 cm, per the ITT set definition.

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The number and proportion of subjects with treatment failure as defined above will be tabulated per treatment group. A 95% confidence interval will be constructed for each proportion. The Cochran Mantel-Haenszel (CMH) test will compare the proportion of treatment-failure between the two groups using the study stratification factors used for randomization 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 hypotheses will be tested for a significant difference in the composite treatment-failure proportions between D-PLEX+SoC (P_{DPLEX}) and SoC (P_{SoC}) treatment:

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

vs.

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

For final analysis, the IA-adjusted significance level will be used. In the case of small number of events (less than 5 events in any study arm), the Fisher exact test will be used.

The number and percent of each of the failure types comprising the primary endpoint will be described by group.

Sensitivity and supportive analyses for the primary endpoint analysis will be defined and detailed in the statistical analysis plan. These will address the following aspects:

- Using the mITT and PP populations
- 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.
- Difference between the treatment and control groups when controlling for possible imbalance in important baseline factors will be analysed by evaluating the odds ratio obtained from estimating a multiple logistic regression model. The list of baseline variables will be finalized in the SAP.
- Sensitivity analysis for the imputation of missing data due to other reason than death (please refer to Section 14.3.7)
- In the event the interim analysis yields a statistically significant result for primary efficacy, and subjects are continued to be enrolled in the study, it should be noted that no future primary endpoint analysis will be conducted as statistical significance and rejection of the null hypothesis will have already occurred. Instead, the primary endpoint measures will be described, and a 95% confidence interval will be provided but no formal statistical testing will be completed.

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14.3.3 Secondary Efficacy Endpoint Analysis

14.3.3.1 Key secondary endpoints

In accordance with the type 1 error control strategy specified for this study, the key secondary endpoints are to be tested once (not part of interim analysis), using an hierarchical approach (as described in section **Error! Reference source not found.**), and based on the Final Analyses and Reporting plan as described in section 14.3.9.

The key secondary endpoints defined for the study are:

1. Infection rate as measured by the proportion of subjects with at least one SSI event, occurring within 30 days post abdominal (index) surgery and determined by a blinded and independent adjudication committee, in the population of subjects with target incision length >20 cm.
2. Infection rate as measured by the proportion of subjects with at least one abdominal target incisional infection event, occurring within 30 days post abdominal surgery and determined by a blinded and independent adjudication committee, in the overall study population (incision ≥ 7 cm). All-cause mortality and re-interventions through the abdominal incision (target) within 30 days post index surgery will be analysed as treatment failure.
3. Number (percent) of subjects with at least 1 score of ASEPSIS > 20 within 30 days post abdominal (index) surgery.

The number and proportion of subjects with SSI event as defined in the 1st key secondary endpoint above, will be tabulated per treatment group. A 95% confidence interval will be constructed for each proportion. A Cochran Mantel-Haenszel (CMH) test will compare the proportion of SSI between the two groups, using the study stratification factors used for randomization, 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). In the case of small number of events (less than 5 events in any study arm), the Fisher exact test will be used. This endpoint will be formally tested only if the primary endpoint is significant, using two-sided 5% significance level.

The number and proportion of subjects with SSI event as defined in the 2nd key secondary endpoint above, will be analyzed similarly as the 1st key secondary endpoint, this time using the all-randomized analysis set. This endpoint will be formally tested only if the 1st key secondary endpoint is significant, using two-sided 5% significance level.

The number and proportion of subjects who reached a score of ASEPSIS > within 30 days post abdominal index surgery will be presented by group. This endpoint will be formally tested only if the 2st key secondary endpoint is significant, using two-sided 5% significance level.

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Missing data strategy for these endpoints is described in section 14.3.7. The primary analysis for these endpoints will be ITT and sensitivity analyses will be defined and detailed in the SAP.

14.3.3.2 Additional secondary efficacy endpoint

Details regarding the analyses of the additional secondary efficacy endpoints will be described in the SAP for the study.

14.3.4 Type 1 error control

The overall study-wise type I error will be 5%.

To protect the study from type I error inflation due to the multiple endpoints, all the key secondary endpoints will be interpreted inferentially only if a statistically significant treatment effect is detected in the primary endpoint (at the interim analysis or at the final analysis). Type I error will be further controlled for the key secondary endpoints by employing the Hierarchical Approach, (i.e., the 2nd key secondary endpoint will be formally analysed only in case the 1st key endpoint will be 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 [REDACTED] subjects (with incision length >20 cm) 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 the 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 number of subjects at interim/final size of the ITT population).

With regard to the unblinded sample size reassessment, 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).

The planned futility analysis that is part of the IA is concerned with stopping for futility and thus inflation of type 1 error is not introduced as well.

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14.3.5 Subgroup analysis

The primary and key secondary endpoints will be analysed by incision length with the following categories: 7 - ≤ 10 cm, >10 - ≤ 20 cm, 7- ≤ 20 cm.

14.3.6 Safety Evaluations

Prior to analysis, all AEs will be coded using the MedDRA coding dictionary.

The number and proportion of subjects reporting at least one treatment-emergent adverse event (TEAE) and the number and proportion of subjects reporting at least one TEAE by MedDRA system organ class (SoC) and preferred term will be reported. This analysis will be repeated for serious AEs (SAEs) and for treatment-related AEs. Additional summaries of TEAEs will be performed by severity and by relationship to treatment.

Vital sign measurements and clinical laboratory parameters will be summarized via descriptive statistics (mean, standard deviation, median, minimum, maximum, and number of non-missing observations) for each post-treatment time point. Shift tables will be constructed for physical exams to display any changes in body system findings over time. Responses to the Modified Vancouver Scar Scale will be summarized with counts and proportions by time point.

Additional analyses of safety will be described in the SAP for the study.

14.3.7 Handling of Missing Data

To be recalled, in the calculation of the primary endpoint (section 14.3.2), 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 will be addressed as treatment failure events in all sensitivity and supportive analyses of the primary efficacy endpoint.

As specified in section 14.3.2, for the purpose of the primary efficacy analysis, based on ITT population, 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 re-intervention in the target incision, will be imputed as treatment failures.

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

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

For the 2nd key secondary endpoint, the missing data strategy defined for the primary endpoint will be applied.

Time to adjudicated SSI during 30 days post index surgery endpoint will handle the missing values as censored.

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.

Full details on sensitivity analyses for handling missing data for the primary endpoints and for the key secondary endpoints will be provided in the SAP.

14.3.8 Interim Analyses

One comparative interim analysis for an early efficacy or futility stop or unblinded sample size re-estimation will be conducted when about a total of [REDACTED] subjects (with incision length > 20 cm) will be 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, as detailed in section 14.1.1. The outcome of this interim analysis will be either early stopping for efficacy or futility, or continuation to planned final ([REDACTED] subjects with incision length > 20 cm) or continue with sample size increased up to maximal size of [REDACTED] only if the comparative result falls in a predefined 'Promising Zone'.

Blinking to comparative interim results

The unblinded interim analysis to be performed by an independent DMC statistician who is otherwise not involved in the study, according to a pre-planned interim analysis plan, and comparative information will be kept confidential within the DMC until the study is unblinded. Moreover, should the Interim analysis result lead to study size continuation/increase, the interim information provided to the sponsor will concern sample size only.

To further safeguard study from potential operational bias, the detailed interim analyses rules will be documented separately, in an access-restricted document.

14.3.9 Final Analyses

Shall the study not be stopped early for efficacy at the interim analysis, all final planned analyses will be performed only after the last subject has completed the study and the database has been locked. Prior the interim analysis, it is planned to lock the primary endpoint efficacy data of those subjects included in the interim analysis (as per the Interim Analysis section above). Only the primary endpoint will be formally tested as part of the IA. In the case of a successful IA result, the positivity of study and formal significance testing of the primary endpoint are to be based on the significant interim analysis result for the primary endpoint,

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and should additional subjects be available in the final full database (i.e. enrolled during the interim analysis period), it will serve to formally assess the key secondary endpoints where the primary endpoint will only be described and presented along with 95% confidence interval. No formal statistical testing will be done on the primary endpoint after the successful result from the interim analysis.

14.4 PHARMACOKINETIC ASSESSMENTS

14.4.1 Pharmacokinetic Analysis

Concentration-time profiles of doxycycline will be analyzed by non-compartmental methods using Phoenix WinNonlin®, Version 8.3.4 or higher, Certara L.P. (Pharsight), St. Louis, MO. Pharmacokinetic parameters and variables will be calculated according to standard equations using actual sampling times relative to dose administration.

Parameter	Definition
$C_{\max}$	maximum observed concentration
$t_{\max}$	the time at which $C_{\max}$ was observed
AUC_{last}	area under the concentration-time curve from time 0 to the time of the last quantifiable concentration before the next dose
C_{last}	last quantifiable concentration
t_{last}	the time at which C_{last} was observed
AUC_{inf}	area under the concentration-time curve from time 0 extrapolated to infinity
λ_z	terminal elimination rate constant
$t_{1/2}$	terminal elimination half-life
CL/F	apparent total body clearance calculated as $\text{dose}/AUC_{\text{inf}}$
V_d/F	apparent total volume of distribution calculated as $(CL/F)/\lambda_z$

Following administration, $C_{\max}$, C_{last} , $t_{\max}$ and t_{last} will be obtained directly from the experimental observations. If multiple maxima occur at equal concentrations, the first temporal value will be taken.

AUC will be calculated using the linear/log trapezoidal rule, using actual elapsed time since dose administration.

The number of data points included in the regression for determination of λ_z and $t_{1/2}$ after single-dose will be determined by visual inspection, but a minimum of 3 data points in the terminal phase, excluding $C_{\max}$, will be required to estimate λ_z . The λ_z values (and consequently $t_{1/2}$, CL/F , V_d/F and AUC_{inf}) will be considered unreliable estimates if the adjusted coefficient of determination ' R_{sq} ' is less than 0.8 and excluded from summaries.

The proportion of AUC_{inf} due to extrapolation (AUC_{extr}) will also be calculated and expressed as a percentage. The value of AUC_{extr} should be less than or equal to 20% to be considered to be

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well estimated. If AUC_{extr} is higher than 20%, then the values of AUC_{inf} , CL/F and V_d/F will be considered unreliable and therefore excluded from the summaries.

14.4.2 Statistical Methods

14.4.2.1 General

Descriptive statistics will be provided for PK parameters by treatment (number of vials of D-PLEX administered), and for concentrations by treatment and nominal time. Descriptive statistics will include sample size (n), mean, median, standard deviation (SD), median, and the coefficient of variation expressed as a percentage (%CV).

14.4.2.2 Analysis Populations

Pharmacokinetic Population: All subjects treated with D-PLEX and for whom the PK data are considered to be sufficient to compute at least one PK parameter for doxycycline (as defined in Section 14.4.1).

14.4.2.3 Analysis of Pharmacokinetic Data

PK parameters will be calculated using non-compartmental methods. PK data will be summarized using descriptive statistics by dose (number of vials of D-PLEX administered) and presented in tabular and graphical form. Individual, mean (+SD), and composite plots of concentration versus time will be constructed.

14.5 NAME OF COMMITTEES INVOLVED IN TRIAL

14.5.1 DATA MONITORING COMMITTEE (DMC)

An independent of the Sponsor/Investigator involved in the study DMC will be utilized in this study to ensure external objective medical and/or statistical review of safety issues in order to protect the ethical and safety interests of subjects and to maintain the scientific validity of the study.

In order to maintain blinding in communication with the DMC, an Independent Biostatistician separate from the study team will be appointed to provide required information to the DMC.

Blinded tables and listings will contain pooled blinded data and will be distributed to PolyPid personnel by the unblinded statistician. Unblinded tables and listings will contain unblinded data and will be distributed only to DMC voting members directly by the unblinded statistician (at the beginning of such e-mails, the unblinded statistician will detail who are the unblinded team members specifically for each case and with whom information can be forwarded to or discussed with).

Data management and clean-up will be done on blinded data sets.

The schedule of any planned interim analysis and the analysis plan for DMC review is described in the charter.

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14.5.2 ENDPOINTS ADJUDICATION COMMITTEE (EAC)

An independent of the Sponsor/Investigator involved in the study and blinded to treatment arm “Endpoint Adjudication Committee” will be established.

This committee is an independent clinical events classification committee, composed of a minimum of 3 experts in the fields of surgeries and infectious disease, who will remain blinded to the treatment assignments.

All information collected regarding efficacy (abdominal wall infection) will be reviewed by the “Endpoint Adjudication Committee”. It is within their scope to make an independent decision whether a subject has met the primary endpoint.

The Committee’s adjudication blinded decision will be forwarded to the Sponsor.

In case of dispute between Investigator assessment of infection and the committee’s assessment, the Endpoint Adjudication Committee’s assessment on primary endpoint evaluation will overrule the Investigator assessment.

15 DIRECT ACCESS TO SOURCE DATA DOCUMENTS

As required, the PI shall obtain permission for direct access to source documents from the subject, hospital administration and national regulatory authorities before starting the clinical trial.

The Investigator will permit trial-related monitoring, audits, EC/IRB review, and regulatory inspection(s), providing direct access to source data/documents. Trial participants are informed of this during the informed consent discussion. Participants will consent to provide access to their medical notes.

16 ETHICS & REGULATORY REQUIREMENTS

The protocol, ICF and Authorization for the Use and Disclosure of Health Information or country specific confidentiality requirements must be reviewed and approved by the respective EC/IRB and the Sponsor before subject enrolment. Changes to the protocol must be approved in writing by the Sponsor and the EC/IRB (as applicable) before the change is implemented.

Prior to subject enrolment, a signed copy of the EC/IRB approval letter addressed to the Investigator must be submitted to the Sponsor, certifying study approval. Investigators are responsible for submitting and obtaining initial approval and continuing approval from the EC/IRB and forwarding copies of the approval letters to the Sponsor. The original letters are to be kept in the investigational centers’ Regulatory Binder designated for this study.

The Investigator will notify the Sponsor immediately of withdrawal of EC/IRB approval.

17 QUALITY CONTROL (STUDY MONITORING)

In accordance with applicable regulations, GCP, and Sponsor’s procedures, the site will be contacted prior to the start of the study to review with the site staff the protocol, study requirements, and their responsibilities to comply with regulatory, ethical, and Sponsor’s requirements. When reviewing

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data collection procedures, the discussion will include identification, agreement and documentation of data items for which the CRF will serve as the source document.

The Investigator and the head of the medical institution (where applicable) agree to allow the monitor direct access to all relevant documents and to allocate their time and the time of their staff to monitor to discuss findings and any issues.

Monitoring visits will be conducted in a manner to ensure that the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

A trial specific monitoring plan will be established for the study. The trial will be monitored according to the agreed plan.

18 QUALITY ASSURANCE (QA)

To ensure compliance with GCP and all applicable regulatory requirements, the Sponsor may conduct a QA assessment and/or audit of the site. Regulatory authorities may conduct a regulatory inspection at any time during or after completion of the study.

The Investigator (and institution) agree to grant the advisor(s), auditor(s) and inspector(s) direct access to all relevant documents and to allocate their time and the time of their staff to discuss the conduct of the study, any findings/relevant issues and to implement any corrective and/or preventive actions to address any findings/issues identified.

19 INSURANCE

The Sponsor will provide insurance or indemnify (legal and financial coverage), the Investigator/ Institution against claims arising from the study, insurance covering the cost of treatment of the subjects in the event of clinical-trial-related injuries, in accordance with national regulations, except for claims that arise from malpractice, negligence, or non-compliance with the protocol. The certificate of insurance and coverage will be provided upon request.

20 PUBLICATION POLICY

All data generated from this study are the property of Sponsor and shall be held in strict confidence along with all information furnished by Sponsor, subject to the right of the Investigator or any member of his/her staff to publish the results in accordance with the publication procedure to be defined in the clinical trial agreement.

Independent analysis and/or publication of this data by the Investigator or any member of his/her staff are permitted subject to obtaining the prior written consent of Sponsor.

Written permission to the Investigator will be contingent on the review by Sponsor of the statistical analysis and manuscript and will provide for nondisclosure of Sponsor confidential or proprietary information. In all cases, the parties agree to submit all manuscripts or abstracts to all other parties

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30 days prior to submission. This will enable all parties to protect proprietary information and to provide comments based on information that may not yet be available to other parties.

21 STATEMENT OF COMPLIANCE

The trial will be conducted in compliance with the approved protocol, Declaration of Helsinki, ICH GCP and local applicable regulatory requirement(s).

The clinical trial shall not begin until the required approval or favourable opinion from the EC/IRB has been obtained and, if applicable, any local or national regulatory authority approvals or notifications have been obtained.

The Sponsor has obtained clinical trial insurance that will cover expenses in the event of any physical injury resulting from research procedures.

22 APPENDICES

[Appendix 1: ASEPSIS Score Assessment](#)

[Appendix 2: Modified Vancouver Scar Scale](#)

[Appendix 3: PK instructions for Collection and Processing](#)

[Appendix 4: Surgical Site Assessment Questionnaire](#)

[Appendix 5: Charlson Co-Morbidity Index](#)

[Appendix 6: Document Change History](#)

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APPENDIX 1: ASEPSIS SCORE ASSESSMENT

ASEPSIS provides numerically score during an inspection of the surgical site. The final score is being interpreted to severity of wound appearance and the clinical consequences of the infection.

Table 1. ASEPSIS wound score outline⁽¹⁾

Points for:	Proportion of wound affected, %					
	0	<20	20 - 39	40 - 59	60 - 79	>80
Serous exudates	0	1	2	3	4	5
Erythema	0	1	2	3	4	5
Purulent exudates	0	2	4	6	8	10
Separation of deep tissues	0	2	4	6	8	10
Additional points for:						
Antibiotic treatment required						10
Drainage of pus under local anaesthetic						5
Drainage of wound under general anaesthetic						10
Isolation of bacteria						10
Stay as inpatient >14 days						5
Total						0 - 70

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APPENDIX 2: MODIFIED VANCOUVER SCAR SCALE

The Modified Vancouver Scar Scale documents the change in scar appearance, through a numeric assessment of four characteristics of the wound.

Lower scores indicate better wound healing.

Modified Vancouver Scar Scale		
Vascularity	Normal	0
	Pink	1
	Red	2
	Purple	3
Pigmentation	Normal	0
	Hypopigmentation	1
	Mixed	2
	Hyperpigmentation	3
Pliability	Normal	0
	Supple	1
	Yielding	2
	Firm	3
	Adherent	4
Height	Flat	0
	1-2 mm	1
	3-4 mm	2
	5-6 mm	3
	> 6 mm	4

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APPENDIX 3: PK INSTRUCTIONS FOR COLLECTION AND PROCESSING

The following instructions must be followed for the collection of plasma PK samples.

1. At the allotted time points, draw at least 4 mL of venous blood into a polypropylene K₂ EDTA lavender top tube. Record time and date of collection for each sample.
2. Gently invert tube at least 8 times to allow the specimen to mix with the anticoagulant in the tube to avoid clotting.
3. Store vacutainer at ambient temperature prior to centrifugation. Proceed to centrifugation within 18 hours of blood draw.
4. Centrifuge the tube(s) at 2000 rpm (equivalent to 3000 g) for 15 minutes.
5. Using a disposable pipette, transfer the supernatant (plasma) layer into 2 pre-labelled polypropylene 2ml Eppendorf tubes. Try to keep equal amounts of plasma with the 2 tubes.
 - Mark these 2 tubes as: A (primary) and B (back-up), complete the appropriate label and place them onto the 2ml tubes.
6. Plasma samples must be stored at -20°C or below.

PK Sample Specimen Handling

1. Two cryotubes (A and B) are obtained for each sample time point. Shipments must be split.
2. It is important that SHIPMENTS MUST NOT CONTAIN BOTH CRYOTUBES A and B for the same time point, in the same shipment.
3. Specimen collection times must be documented in the source records. These must be kept up to date.
4. Cryotubes should be packed in groups with samples A and B packed separately, according to subject number and stored at or below -20°C prior to shipment to: ICON Laboratories, Inc.

Back-up Sample Shipment

Back-up samples are to be shipped in line with instructions from the study CPM. ICON Laboratory will send an e-mail alert to the site confirming receipt of the primary samples. Back-up samples should be handled and shipped in the same manner as the primary samples.

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APPENDIX 4: SURGICAL SITE ASSESSMENT QUESTIONNAIRE

	Y	N
Any symptoms detected?		
If yes, please enter Start Date of first symptom: ____ / ____ / ____		

Localized pain		
Tenderness		
Localized swelling		
Localized Heat		
Fever (>38°C)		
Erythema (If yes, please mark the % of wound affected)		
<20% 20%-39% 40%-59% 60%-79% >80%		
Separation of deep tissues (If yes, please mark the % of wound affected)		
<20% 20%-39% 40%-59% 60%-79% >80%		

Is there a <u>drainage (exudates)</u> from the target incision (If yes, please mark the % of wound affected)		
I- Serous drainage?		
<20% 20%-39% 40%-59% 60%-79% >80%		
II- Purulent drainage?		
<20% 20%-39% 40%-59% 60%-79% >80%		

Involves skin and subcutaneous tissue of the incision		
Involves deep soft tissues of the incision (e.g., fascia and muscle layers)		
<u>Organisms identified</u> by microbiologic testing method		

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Superficial incision that is <u>deliberately opened</u> (i.e. removal of pins to better examine the incision)		
A deep incision that <u>spontaneously dehisces</u> , or is <u>deliberately opened</u> or aspirated by a physician		
Drainage of pus from the wound under local anesthetic		
Drainage of pus from the wound under general anesthetic		
An abscess or other evidence of infection involving the deep incision that is detected on gross anatomical or histopathologic exam, or imaging test		
Antibiotic treatment required (due to incision infection)		
Stays as inpatient > 14 days (due to incision infection)		
<u>Diagnosis</u> of a Superficial incisional SSI <u>by the assessor</u>		

Final Assessor Decision

	Yes	No
Superficial Surgical Site Infection		
Deep Surgical Site Infection		

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APPENDIX 5: CHARLSON CO-MORBIDITY INDEX (CCI)

Predicts 10-year survival in patients with multiple comorbidities.

Medical Condition	Score
Age	
<50 years	0
50–59 years	+1
60–69 years	+2
70–79 years	+3
≥80 years	+4
Myocardial infarction	
History of definite or probable MI (EKG changes and/or enzyme changes)	
No	0
Yes	+1
Chronic Heart Failure	
Exertional or paroxysmal nocturnal dyspnea and has responded to digitalis, diuretics, or afterload reducing agents	
No	0
Yes	+1
Peripheral vascular disease	
Intermittent claudication or past bypass for chronic arterial insufficiency, history of gangrene or acute arterial insufficiency, or untreated thoracic or abdominal aneurysm (≥6 cm)	
No	0
Yes	+1
CVA or TIA	
History of a cerebrovascular accident with minor or no residua and transient ischemic attacks	
No	0
Yes	+1
Dementia	
Chronic cognitive deficit	
No	0
Yes	+1
COPD	
No	0
Yes	+1
Connective tissue disease	
No	0
Yes	+1
Peptic ulcer disease	
Any history of treatment for ulcer disease or history of ulcer bleeding	
No	0
Yes	+1

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Medical Condition	Score
Liver disease Severe = cirrhosis and portal hypertension with variceal bleeding history, moderate = cirrhosis and portal hypertension but no variceal bleeding history, mild = chronic hepatitis (or cirrhosis without portal hypertension) None Mild Moderate to severe	0 +1 +3
Diabetes mellitus None or diet-controlled Uncomplicated End-organ damage	0 +1 +2
Hemiplegia No Yes	0 +2
Moderate to severe Chronic Kidney Disease Severe = on dialysis, status post kidney transplant, uremia, moderate = creatinine >3 mg/dL (0.27 mmol/L) No Yes	0 +2
Solid tumor None Localized Metastatic	0 +2 +6
Leukemia No Yes	0 +2
Lymphoma No Yes	0 +2
AIDS No Yes	0 +6
Is this a COVID-19 patient? For research purposes only; answer does NOT impact results. Confirmed positive Suspected Unlikely Confirmed negative	

Summary score	
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APPENDIX 6: DOCUMENT CHANGE HISTORY

Version 07	Title and Investigator signature page – EudraCT number was change to EU CT number	Updating the EU CT number in line with Regulation (EU) No 536/2014 (CTR)
Version 06	Section 9.1.12, 9.2.3, Table 1 – Pregnancy test timelines	Consolidated protocol for transition of the clinical study in line with Regulation (EU) No 536/2014 (CTR).
Version 05	Summary and throughout the document, Section 8.1 - Study population was updated to enrich study population with subjects with incision length more than 20 cm	Study population was updated to enrich study population with subjects with incision length more than 20 cm following the D-PLEX 311 study results and FDA recommendation.
	Summary, Sections 8, 14 – Primary endpoint analysis population was updated following the updates of the study population to subjects with target incision >20 cm	Primary endpoint analysis population was updated following the updates of the study population to subjects with target incision >20 cm, per updated ITT set definition.
	Summary, Sections 7.2, 14.3 – Additional key endpoint was added to evaluate the overall population with incision length ≥7 cm	Additional key endpoint was added to evaluate the overall population with incision length ≥7 cm, as the Primary endpoint was changed.
	Summary, Sections 8, 14 - Number of subjects in the study was updated	Number of subjects in the study was updated according to updated population and results of D-PLEX 311 study.
	Summary, Sections 8, 9.2.2 - Standard of Care options were re-phrased	Standard of Care options were re-phrased to be clearer.
	Summary, Sections 8, 9.2.2 -3 rd generation of Cephalosporines was added to the SoC antibiotic prophylaxis	3 rd generation of Cephalosporines was added to the SoC antibiotic prophylaxis following updating standard of practice.

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	Summary, Sections 8, 9.1.20, Appendix 3 - Doxycycline pharmacokinetic substudy was added	Doxycycline pharmacokinetic sampling and testing in the selected study sites were added to assess the pharmacokinetic profile of D-PLEX applied during abdominal surgeries with incision length >20 cm.
	Summary, Section 14.1.1 – An unblinded interim analysis for an early efficacy or futility stops or unblinded sample size re-estimation was added	One comparative interim analysis for an early efficacy or futility stops or unblinded sample size re-estimation was added instead of blinded sample size re-estimation.
	Summary, Section 14.1.1 - A blinded sample size re-estimation was cancelled	A blinded sample size re-estimation was cancelled as unblinded interim analysis was added.
	Summary, Section 14.1.1 - The significance level for the primary end point analysis was updated	The significance level for the primary end point analysis was updated following a planned unblinded Interim analysis.
	Summary, Section 8.4 – Inclusion criterion # 1 updates	Inclusion criterion #1 was updated following the update of the study population.
	Summary, Section 8.4 – Inclusion criteria # 2 updates	Eligibility criteria were updated to determine the new study population.
	Summary, Section 8.5 – Exclusion criteria #4 updates	Exclusion criteria was updated to exclude subjects that any additional procedure is planned during the colorectal resection.
	Summary, Section 8.5 – Exclusion criteria #6 updates	Exclusion criteria was updated to exclude subjects who received radiation to pelvis area.
	Summary, Section 8.5 – Exclusion criteria #16 updates	Exclusion criteria #16 was re-phrased to be clearer.
	Summary, Section 14.2.1 - “All-enrolled population” definition was added	“All-enrolled population” definition was added to describe overall population in the study, that will include all randomized subjects regardless of their incision length.

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	Summary, Section 14.2 - ITT population was updated	ITT, mITT and Safety populations definitions were updated following the updated study population
	Section 5.3 – Addition of the D-PLEX 311 study results	Clinical data was updated with the results of the D-PLEX 311 study (Phase 3)
	Section 5.6 – updates of the potential risks	Potential risks were updated according to the current DSUR, that was updated following the results of D-PLEX 311 study.
	Sections 9.1.3, 9.1.12 - Randomization process was updated	Randomization process was updated with Sponsor approval request to control the subject's recruitment process.
	Summary, Section 9.1.9, 10.6, 10.8, 10.9, 10.12 - Adverse Events for Special Interest (AESI) were added	Adverse Events for Special Interest (AESI) were added to AE reporting request to add the more closed reporting of the AE related to impaired wound healing
	Section 9.1.6 - CDC definition of the SSI was updated	CDC definition of the SSI was updated according to the last version of CDC (Jan 2023)
	Section 9.1.7 - Management of SSI was clarified	Management of SSI was clarified to be clearer
	Section 9.1.11 - HbA1C blood test analysis was added	HbA1C analysis was added to the Central lab blood tests during the Screening to reveal subjects with diabetic signs
	Section 9.1.12 - Urinalysis was cancelled	Urinalysis was cancelled following the results of D-PLEX 311 (phase 3) study
	Section 9.1.13 - Bacteriological testing process clarification	Bacteriological testing process was clarified to provide sites with more detailed instructions
	Table 1 – Study assessment was updated	Table 1 study assessment was updated according to the study design updates
	Section 10.1.3 - Product Handling was updated	Product Handling was updated following FDA comments
	Section 10.2.1 - Permitted concomitant medications updates	Concomitant medications permitted during the study were updated to be clearer

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	Section 10.2.2 – Prohibited concomitant medication updates	Concomitant medications prohibited during the study were updated to be clearer
	Section 14.4.2 – Definition and responsibility of EAC were updated	EAC definition and responsibility were updated according to the updated adjudication process.
Version 04	Section 10.1.3 – Instruction for use was updated	Instruction for use was updated following FDA's feedback.
	Summary, Exclusion criteria, Sections 8.4, 8.5– clarification and re-wording of Exclusion criteria #1	Exclusion criteria #1 were slightly clarified and re-worded to better clarify it to the study site team
Version 03	Summary, Sections 8.4, 8.5 – Inclusion criteria # 1 was updated and Exclusion criteria #1 was added to include only subjects scheduled for elective surgery.	Eligibility criteria were updated to exclude subjects who were scheduled for urgent or emergency surgeries as was planned prior to COVID-19 pandemic.
	Summary, Section 8.5 – Exclusion criteria #2 was added to exclude subjects that were operated within last 6 months.	Exclusion criteria was updated to exclude subjects that were operated within last 6 months, as this may compromise the evaluation of the incision healing.
	Summary, Section 8.4 - Inclusion criteria #2 was clarified with thresholds of vital signs (blood pressure, heart rate and temperature)	Clarification of the preoperative hemodynamically stable definition
	Summary, Section 8.5 – Exclusion criteria #12 was added to exclude subjects with confirmed severe hepatic impairment	Severe hepatic impairment was separated from the exclusion criteria #17, that includes “other concurrent severe and/or uncontrolled medical condition” according to an EU competent authority request.
	Summary, Section 8.5 – Exclusion criteria #15 was re-phrased to be clearer	Clarification
	Section 5.6 - Potential risks and mitigations due to COVID-19 pandemic were added	In compliance with the EU regulatory requirements and updated EU guideline Guidance on the Management of Clinical Trials During the Covid-19 (Coronavirus)

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		Pandemic
	Section 9.2.1 and Table 1 – pregnancy test timeline was corrected	Pregnancy test timeline was corrected to 1 day before surgery instead of 7 days according to an EU competent authority request.
	Section 8.6 – WOCBP definition was added	Clarification
	Title pages – addition of EudraCT and IND numbers	EudraCT and IND numbers were added to reflect the US and EU investigational study numbers
	Section 8.6 – clarification of contraception timeline	Clarification
	Section 9.1.4 – clarification of blindness	Clarification
	Section 9.1.5 – Emergency Unblinding process was clarified	Clarification
	Section 9.1.7 – SSI reporting process and timelines, and EAC decision were clarified	Clarification
	Section 9.1.9 – AE reporting process and timelines were re-phrased for clarification	Clarification
	Section 9.1.16 and 10.2.2.2 – clarification of high dose of steroids	Clarification
	Section 9.1.18 – correction of typo	Typographical error
	Section 10.1.3 – Instruction for use was updated	Instruction for use was updated following risk assessment that was conducted following the FDA's feedback.
	Section 10.7.2 – Causality definition was corrected	Clarification
	Section 10.8 and 10.9 – SAE and SSI reporting process and	Clarification

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	timelines were updated	
	Section 10.10 and 10.11– SUSAR and DSUR reporting process were re-phrased	Clarification
Version 02	Summary, Sections 8.4, 8.5 - Inclusion/Exclusion criteria updates	Inclusion/Exclusion criteria updated following discussions with KOLs and study Investigators
	Summary, Sections 8.1, 9.1.3 – Clarification of the stratification factors	Stratification factors were clarified due to FDA request
	Sections 9.1.16, 9.2.1, Table 1, Appendix 5 - addition of the Charlson co-morbidity index	Charlson co-morbidity index was added due to FDA request to assess the subject baseline status.
	Summary, Section 9.1.9 – Pregnancy Follow-up clarification	Pregnancy reporting and Follow-up was clarified as was previously missing by mistake.
	Section 9.1.18 – addition of cellular picture to surgical site assessment.	Investigator is asked to photocopy the incision area to be uploaded to a save location as aiding tool for adjudication committee.
	Section 9.1.16 – Medical History and Concomitant Medication collection was updated	Medical History and Concomitant Medication collection was updated to be clearer to Investigators and study site's staff.
	Section 10.1.3.3 - Product Preparation process was updated	Product Preparation process was updated to be clearer to Investigators.
	Section 8.1 – Incision length typo mistake was corrected	Typo mistake of incision length was corrected.
	Summary, Sections 8.1, 9.2.2 - pre-defined SoC requirement was updated	Pre-defined SoC requirement was updated to be clearer to Investigators and study site's staff.
	Summary, Sections 14.3.7 – clarification regarding deaths and re-interventions	Deaths and re-interventions analyses were clarified due to FDA request

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	Summary, Sections 14.2.3 – mITT population definition was updated	Definition of mITT population was updated due to FDA request
	Summary, Sections 14.3.2, 14.3.3 - Mantel–Haenszel clarifications	Mantel–Haenszel test analysis was clarify due to FDA request and SAP correction
	Sections 14.3.3 – Secondary endpoint typo mistake was corrected	Secondary endpoint typo mistake was corrected to be consistent throughout the whole protocol
	Summary, Section 14.1.1 – "re-assessment" was replaced with "re-estimation"	Word "re-assessment" was replaced with "re-estimation" to be consistent through the protocol.
	Some words were corrected to be consistent through all document	"Patient" was replaced with "Subject" to be consistent through all document.
Version 01	NA	First Issue

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Document Signatures

Participant Name	Participant Type	Action	Task Performed On
	Approver	Send to QA Approval	2024-09-25 13:08
	Approver	Send to QA Approval	2024-09-25 13:10
	Approver	Send to QA Approval	2024-09-25 13:22
	Approver	Send to QA Approval	2024-09-25 13:54
	Approver	Send to QA Approval	2024-09-25 15:03