D-PLEX 311 Clinical Trial Protocol

Legacy Document Number: CL-0027

Study Number:	**Study D-PLEX 311**
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 06**
EudraCT Number	2020-002325-28
IND Number	131060
Sponsor	PolyPid Ltd, 18 HaSivim Street, Petach Tikva, Israel, 4959376 Tel: +972-74-719-5700
Sponsor Protocol Number	Study D-PLEX 311
Phase of Trial	Phase III
Site(s)	About [redacted] centers in the US, Europe and Israel
Sponsor Representative	[redacted] Chief Scientific Officer PolyPid Ltd. 18 HaSivim Street, Petach Tikva, 4959376 Israel Tel: [redacted] Email: [redacted]

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SPONSOR SIGNATURE PAGE

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Protocol Version:	**06**
EudraCT Number:	2020-002325-28
IND Number:	131060
Sponsor:	PolyPid Ltd. 18 Hasivim Street, Petach Tikva, 4959376, Israel Tel: [redacted]
Sponsor Representative Name:	[redacted] Chief Scientific Officer PolyPid Ltd.
Title:	CSO
Signature:	[redacted]
Date:	21

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INVESTIGATOR SIGNATURE

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IND Number:	**131060**

I have read and understood 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.

Principal Investigator (print name)

Principal Investigator (signature) *Date*

POLYPID
OPTIMIZED THERAPEUTICS

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DOCUMENT HISTORY

Revision	Date reviewed/revised	Changes
Version 01	NA	First Issue
Version 02	30 Dec 2019	• Changes in study design due to re-evaluation of the overall infection rate in Europe and US regions • Implementation of HPRA recommendations.
Version 03	05 May 2020	Changes in study design due re-evaluation of overall infection rate in Europe and US regions and FDA recommendations.
Version 04	19 Aug 2020	Changes in study design following FDA review of the protocol.
Version 05		Changes in study design following study procedures clarifications, EU regulatory authorities requests harmonized in this version.
Version 06		Update of study end points and sample size calculation as agreed with FDA

DOCUMENT CHANGE HISTORY

(sections numbering to be amended following corrections in the doc body)

Revision	Changes	Reason for changes
Version 06	Summary, Sections 8.3, 14.1 – update of the sample size calculation	Number of subjects planned to be recruited to the study was updated due to re-estimation of initial assumptions
	Summary, Inclusion 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
	Summary, Sections 7.1, 7.2.2, 9.1.6, 14.3.2- Update of primary endpoint analysis definition by moving the Re-interventions in the target incision within 30 days post-	Primary endpoint analysis definition was updated according to the updated study assumptions that were defined before COVID pandemic.

POLYPID
OPTIMIZED THERAPEUTICS

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Revision	Changes	Reason for changes
	surgery from the primary end point to the secondary end points	
	Summary, Section 14 – Unblinded interim analysis was added to the study design	Unblinded interim analysis for an early efficacy or futility stopping or unblinded sample size re-estimation was added to the study design to re-estimate the study sample size that was defined before COVID pandemic.
	Summary, Sections 7.1, 7.2.2 – clarification of the re-intervention definition	Re-intervention definition was clarified to be more precise
	Section 10.1.3 – Instruction for use was updated	Instruction for use was updated following FDA's feedback.
	Summary, Section 14 – updates of the sample size calculation	Sample size calculation was updated according to the updated assumptions

1 TABLE OF CONTENTS

2 LIST OF ABBREVIATIONS

2.1 **AE -** Adverse Event

2.2 **ASEPSIS - A**dditional treatment, **S**erous discharge, **E**rythema, **P**urulent exudate, **S**eparation of deep tissue, **I**solation of bacteria, **S**tay duration as inpatient

2.3 **ALT(SGPT) -** Alanine Aminotransferase

2.4 **AST(SGOT) -** Aspartate Transaminase

2.5 **AUC –** Area Under Curve

2.6 **BMI -** Body Mass Index

2.7 **B-TCP -** β- Tri-Calcium Phosphate

2.8 **BP –** Blood Pressure

2.9 **CDC -** Center for Disease Control

2.10 **CDMS -** Clinical Data Management System

2.11 **CKD -** Chronic Kidney Disease

2.12 **CM -** Centimetres

2.13 **CRF -** Case Report Form

2.14 **ECRF -** Electronic Case Report Form

2.15 **CRP -** C-Reactive Protein

2.16 **CVA -** Cerebro Vascular Accident

2.17 **DMC -** Data Monitoring Committee

2.18 **DSUR –** Development Safety Update Report

2.19 **DSSI -** Deep Surgical Site Infection

2.20 **EA -** Enterobacter Aerogenes

2.21 **EC/IRB -** Ethics Committee/Independent Review Board

2.22 **ESRD -** End Stage Renal Disease

2.23 **EU -** European Union

2.24 **G -** Gram

2.25 **GCP -** Good Clinical Practice

2.26 **GMP -** Good Manufacturing Practice

2.27 **HR –** Heart Rate

2.28 **IAP -** Interim Analysis Plan

2.29 **IB -** Investigator's Brochure

2.30 **ICF -** Informed Consent Form

2.31 **ICH -** International Council for Harmonisation Of Technical Requirements For Pharmaceuticals For Human Use

2.32 **IP -** Investigational Product

2.33 **IPAL -** Investigational Product Accountability Log

2.34 **ISO -** International Organization for Standardization

2.35 **ITT -** Intention to Treat

2.36 **IV -** Intravenous

2.37 **IWRS -** Interactive Web Randomization System

2.38 **LDH -** Lactate Dehydrogenase

2.39 **MG -** Milligram

2.40 **MIC -** Minimal Inhibitory Concentration

2.41 **MITT -** Modified Intention To Treat

2.42 **MOH -** Ministry Of Health

2.43 **MRSA -** Methicillin-Resistant Staphylococcus Aureus

2.44 **NHSN -** National Healthcare Safety Network

2.45 **NIS -** National Inpatient Sample

2.46 **OR -** Operation Room

2.47 **PI -** Principal Investigator

2.48 **PK -** Pharmacokinetics

2.49 **PLEX -** Polymer-Lipid Encapsulation Matrix

2.50 **PP -** Per Protocol

2.51 **PT -** Preferred Term

2.52 **QA -** Quality Assurance

2.53 **QC -** Quality Control

2.54 **RBC -** Red Blood Cell

2.55 **SAP -** Statistical Analysis Plan

2.56 **SAR -** Serious Adverse Reaction

2.57 **SAE -** Serious Adverse Event

2.58 **SOC -** Standard of Care

2.59 **SOP -** Standard Operating Procedure

2.60 **SSI -** Surgical Site Infection

2.61 **SSSI -** Superficial Incisional Surgical Site Infection

2.62 **SUSAR -** Suspected Unexpected Serious Adverse Reaction

2.63 **TEAE -** Treatment Emergent Adverse Event

2.64 **TIA -** Transient Ischemic Attack

2.65 **WBC -** White Blood Cell

2.66 **WOCBP -** Women of Childbearing Potential

3 TRIAL PERSONNEL

3.1 SPONSOR'S REPRESENTATIVES

- [redacted]
  PolyPid Ltd.
  18 Hasivim Street,
  Petach Tikva, 4959376
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4 SUMMARY

Study Number:	Study D-PLEX-311
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:	• 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 [redacted] 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. 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:	Minimum of 950 subjects will be enrolled and randomized into the study, with a maximum of 1400 subjects. The final number of subjects will be determined following a comparative interim analysis for an early efficacy or futility stop or unblinded sample size re-estimation: when about 750 subjects will complete their 30 days (1 month) follow-up and evaluated for primary endpoint.

Test Product:	D-PLEX is a new formulation of extended release of Doxycycline. Each 5g D-PLEX vial contains [redacted] Doxycycline free base ([redacted]), which is equivalent to [redacted] Doxycycline hyclate [redacted]). D-PLEX is supplied as a sterile powder to be reconstituted to paste in the operating room using standard techniques. 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 international guidelines[3]. 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 mechanical bowel preparation in the preceding several days. Each site will use the same pre-defined SoC for all its subjects during the study. IV antibiotic treatment will be composed of the 1st or 2nd generation of Cephalosporin family plus Metronidazole given within 60 minutes prior to surgery and should be discontinued maximum 24-hour post-surgery. In case of allergy to the Cephalosporin/ Penicillin families or Metronidazole, other IV antibiotic may be used. Pre-operation prophylactic oral antibiotic is not allowed. Mechanical bowel preparation (use of a laxative (polyethylene glycol, sodium phosphate, or a magnesium citrate–based regimen) or a high-volume enema) will be at the discretion of the PI per each site's SOP. Subjects will be stratified to 2 cohorts according to usage of mechanical bowel preparation prophylactic care (Yes or No) and by region (US or Europe + Israel).
Dose Administration:	D-PLEX dose is individualized, pending length of the abdominal target incision, 2-3 vials (5g each, a total max of 15g) in a single

	application. 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).
Duration of Dosing:	D-PLEX is administered as a single application. The active ingredient (Doxycycline) is continuously released for approximately 4 weeks.
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 that is > 10 cm (target incision). Subjects who meet the inclusion criteria and none of the exclusion criteria and who provide a signed Informed Consent Form will be enrolled in the study. Subjects will be randomized into either the investigational arm (D-PLEX + SoC) or to the control arm (SoC only) in a 1:1 ratio. Subjects will be stratified by type of SoC prophylactic antibiotic regimens (prophylactic IV antibiotic with mechanical bowel preparation or prophylactic IV antibiotic without 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.

	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 reintervention occurs. Subjects randomized to the control arm will be treated only with prophylactic IV antibiotic according to SoC. Pre-operation prophylactic oral antibiotic is not allowed. Mechanical bowel preparation will be at the discretion of the PI per each site's SOP (see also section 8.1). For both arms the prophylactic SoC will be consistent and standardized for all sites in the clinical study. 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 all randomized subjects in these sites (sites from Czech Republic will not participate in the PK sub-study). About [redacted] series of [redacted] samples per set will be analyzed. About [redacted] series will be analyzed from subjects receiving 2 vials, about [redacted] series will be analyzed from subjects receiving 3 vials. 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 for an overall of 60 days for safety. <u>Primary Efficacy Endpoint:</u> ● 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 independent adjudication committee. [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-intervention at the primary incision site (target) due to suspected SSI or due to poor wound healing, including wound dehiscence (as verified by the blinded adjudication committee), within 30 days post index surgery will be analysed as treatment failure. Reinterventions for other reasons likely unrelated to the initial treatment (e.g. anastomosis leaks, intraabdominal hemorrhage, bowel obstruction), will not be considered as treatment failure in the primary analysis of the primary endpoint. These events will be considered as treatment failure in a sensitivity analysis. Reintervention is defined as re-opening of the surgery incision, used for the original index surgery, in the operation room (OR). An independent and blinded adjudication committee will review each case suspected for SSI and will adjudicate if meets the SSI endpoint criteria. <u>Secondary Efficacy Endpoints:</u> <u>Key endpoints:</u> ● Infection rate as measured by the proportion of subjects with at least one abdominal target incisional infection event only, occurring within 30 days post abdominal index surgery and determined by a blinded and independent adjudication committee. ● Number (percent) of subjects with at least 1 score of ASEPSIS > 20, within 30 days post abdominal index surgery. <u>Additional endpoints:</u> ● 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-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-surgery. Re-intervention is defined as re-opening of the surgery incision, used for the original index surgery, in the operation room (OR). *SSI related Additional Endpoints*: ● Number (percent) of subjects with adjudicated SSI where at least one Doxycycline-resistant bacteria has 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-surgery that was treated by IV antibiotic. ● Number of IV antibiotic treatment days, administered to subjects who experienced adjudicated SSI during 30 days post-surgery. ● Average of subjects' cumulative ASEPSIS assessment score (AUC) for subjects with adjudicated SSI during 30 days post-surgery. ● 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 2020, 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 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 of 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 that is > 10cm (target incision). 2. Subjects are preoperative hemodynamically stable (BP≤180/110 and ≥90/60 mmHg, and HR≤100 and ≥60 bpm, and temperature ≤38.5^{0}C and ≥35.5^{0}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
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 (ex. toxic

	colitis, ileus/sub-ileus, megacolon, diverticulitis, volvulus, ect.) 2. Subjects who underwent an intra-abdominal surgery within 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. 4. Subjects undergoing concomitant major procedures in addition to the abdominal surgery, including concomitant repair of ventral hernia. Salpingo-oophorectomy and cholecystectomy are allowed. 5. Subjects who received any anti-cancer treatment within the last 4 weeks of surgery. 6. Subjects who received radiation for colorectal cancer to the abdomen area, prior to 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.

	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, other than resectable non-metastatic colorectal cancer, that is the reason for the index surgery, or carcinoma in situ (or other cancer "in situ = Stage 0"), or squamous cell carcinoma of the skin, or basal cell carcinoma of the skin, or a malignancy that has not been in complete clinical remission and without maintenance chemo or immunotherapy for at least 3 years. 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 age 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 study will enrol a minimum of 950 subjects, with 475 subjects allocated to each treatment group. The sample size calculation was based on powering the study with respect to the primary efficacy endpoint. It was assumed that the SSI rate in the SOC arm would as high as 16% range. The study predefined assumption is that D-PLEX +SOC will have effect of 50% on this rate (halving infection rate) relative to SOC alone, thus 8% infection rate in D-PLEX+SOC arm. Assuming rate equal to [redacted] for

	both arms with regard to 30-day treatment failure due to all-cause mortality or re-intervention in the target incision due to suspected SSI or due to poor wound healing, including wound dehiscence, the assumed treatment failure rates were [redacted] and [redacted], for SOC and DPLEX+SOC, respectively. It was calculated that a sample of 882 total subjects provides 90% power to detect the assumed difference in treatment failure rates. The calculation was based on a chi square test, and to account for the planned interim analysis for efficacy (to be performed with two-sided α=0.01), a conservative two-sided α=0.04 level of significance was employed. In order to account for an anticipated about 5% lost-to-follow-up rate, a minimum of 950 subjects (475/treatment arm) will be enrolled. One comparative interim analysis for an early efficacy or futility stop or unblinded sample size re-estimation will be conducted when about 750 subjects will complete the 1-month follow-up and evaluated for the primary endpoint. The interim analysis will include a group sequential design stopping rule for efficacy or futility, combined with unblinded sample size re-assessment based on the 'Promising Zone' approach. The outcome of this interim analysis will be either early stopping for efficacy or futility, or continuation to planned final (950 subjects) or continue with sample size increased up to maximal size of 1400 only if the comparative result falls in a predefined 'Promising Zone'. The interim decision will be made by the independent DMC according to a pre-planned interim analysis plan, and comparative information will be maintained confidential within the independent statistician until the study finally unblinded.
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 type of SoC prophylactic antibiotic regimens (prophylactic IV antibiotic with mechanical bowel preparation or prophylactic IV antibiotic without mechanical bowel preparation) and by region (US or Europe + Israel).
Statistical Methods:	The following analysis populations will be defined for the study: **Intention to Treat (ITT)**: The ITT analysis set will consist of all subjects 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 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 due to suspected SSI or due to poor wound healing, including wound dehiscence).

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.

The analysis of the primary and key secondary efficacy endpoints will be performed on the ITT, mITT and PP populations, with the ITT population serving as the primary analysis set. Analyses of additional 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 abdominal surgery, or all-cause mortality by 30 days. Also re-interventions at the

	primary incision site due to suspected SSI or due to poor wound healing, including wound dehiscence, as determined by a blinded and independent adjudication committee, within 30 days post index surgery and indeterminate outcome (due to missing data) will be considered a treatment failure for the primary ITT analysis. 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 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 for this test will be 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) 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 analysis:	One un-blinded comparative interim analysis will be conducted after a total of about N=750 subjects have been assessed for the primary efficacy 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 futility, or continuation to planned final (950 subjects) or continue with sample size increased up to maximal size of 1400 only if the comparative result falls in a predefined 'Promising Zone'.

5 INTRODUCTION

5.1 BACKGROUND

5.1.1 Background Information on The Product

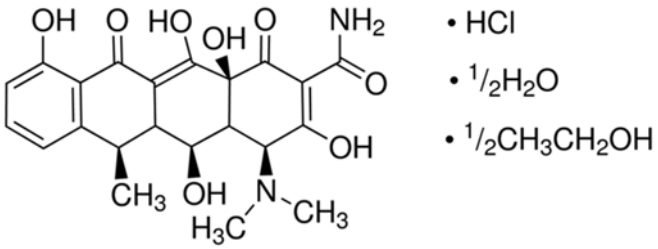
5.1.1.1 Product Name

D-PLEX (Doxycycline/Polymer-Lipid Encapsulation MatriX)
5g D-PLEX per vial contains [redacted] w/w Doxycycline hyclate equivalent to [redacted] ([redacted]) 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-(4α,4aα,5α,5aα,6α, 12aα)]- Doxycycline Hyclate chemical structure is shown in Figure 1 below.

Figure 1: Chemical Structure of Doxycycline Hyclate



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

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

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OPTIMIZED THERAPEUTICS

D-PLEX 311 Clinical Trial Protocol

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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
5 – ≤10	1*
>10 - ≤20	2
>20	3

**will not be used during this study*

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.

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 hysterectomies[3], 30.0%[4] of colorectal surgeries and approximately 6.8% in urological procedures[5]. Chemotherapy and other immuno-suppressive medications affect the function of the immune system and have the potential to increase the SSI rate[6].

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-threatening[7].

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 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 ex-change 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 infection[13–16]. 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 recommended[17]. 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 four weeks following administration. This is the period in which most of the laparotomy colon surgical site infection occur[8]. 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.

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 feacium (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 (>30µg/mL). This is followed by constant Doxycycline concentrations in the range of approximately ~ 3-5µg/mL that is maintained for approximately 30 days. These levels are well above the MIC of the sensitive strain, Staphylococcus aureus (MIC of 1µg/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:

5.2.1 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.

5.2.2 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.

5.2.3 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 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

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 enrolled a total of 201 subjects, that were scheduled for elective abdominal colon surgery involving resection and including at least 1 incision that is ≥ 5cm.

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 citation[18].
- 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 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 citation[18].

These results are highly clinically meaningful and statistically significant, and therefore support the robustness of D-PLEX in abdominal SSI prevention. 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 given it is being gradually released over a period of approximately 4 weeks, 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 a phase II clinical study which was recently completed. It shows detectable plasma levels of Doxycycline for well into four weeks 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.

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, 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.

Potential of bacteria resistant to Doxycycline-treatment failure/ increased severity of infection.
Mitigation:

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

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 0.

Inadequate antibacterial activity of the product

Mitigations:

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

Re-use of D-PLEX between different subjects, can lead to cross contamination.

Mitigations:

- D-PLEX vial will be re-allocated to a different subject only if (i) package carton was never opened (ii) storage conditions were kept and (iii) with the permission of the site's head of pharmacy. If, for some reason, it was not used also after the 2nd allocation - any remaining drug not applied should be discarded.
- Training of site clinical staff.

Photosensitivity reaction: exaggerated sun burn reaction.
Mitigations:

- Close follow up of subjects

Transient hypercalcemia

Mitigation:

- Close follow up of subjects.

Blood levels of calcium are tested at screening and every visit after the procedure during the clinical study.

5.6.1 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 photocopy 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.

5.7 SAFETY & EFFICACY SUMMARY

Based on the current data available from 2 clinical studies with D-PLEX, in which 99 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 treatment 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 surgery and determined by a blinded and independent adjudication committee.
The blinded and independent adjudication committee review will include all index surgery incisions, regardless of any re-intervention at the target site (i.e. re-opening of the surgery incision, used for the original index surgery), for the determination of infection status. The committee will also assess re-interventions at the primary incision site through the abdominal incision (target) for the determination of re-intervention due to suspected SSI or due to poor wound healing, including wound dehiscence. Such event as well as all-cause mortality within 30 days post index surgery will be analysed as treatment failure.

Reinterventions for other reasons likely unrelated to the initial treatment (as determined by the blinded independent adjudication committee; e.g. anastomosis leaks, intraabdominal hemorrhage, bowel obstruction), will be not be considered as failures in the primary analysis of the primary endpoint.

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

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

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

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 2020, chapter 9).

7.2 SECONDARY EFFICACY ENDPOINTS

7.2.1 Key endpoints:

- Infection rate as measured by the proportion of subjects with at least one abdominal target incisional infection event only, occurring within 30 days post abdominal 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)].
- Number (percent) of subjects with at least 1 score of ASEPSIS > 20 within 30 days post abdominal 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 post-surgery during 30 days post index surgery.
- Number (percent) of subjects, re-admitted during 30 days post-surgery (for any reason) and 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-surgery. Re-intervention is defined as re-opening of the surgery incision, used for the original index surgery, in the operation room (OR)

7.2.2.1 SSI related Additional Endpoints:

- Number (percent) of subjects with adjudicated SSI where at least one Doxycycline-resistant bacteria has grown during the bacteriology tests.

- 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-surgery that was treated by IV antibiotic.
- Number of IV antibiotic treatment days administered to subjects experienced adjudicated abdominal incisional SSI during 30 days post-surgery.
- Average of subjects' cumulative ASEPSIS assessment score (AUC) for subjects with adjudicated SSI during 30 days post-surgery.
- Number (percent) of subjects with at least 1 score of ASEPSIS > 20 in subjects with 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 that is > 10 cm. 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. 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 type of SoC prophylactic antibiotic regimens (prophylactic IV antibiotics with mechanical bowel preparation or prophylactic IV antibiotics without 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 confident 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.

D-PLEX will not be re-administered if any reintervention occurs. Reintervention is defined as re-opening of the surgery 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 and is composed of IV antibiotic immediately prior to surgery. 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 or 2nd generation of Cephalosporin family plus Metronidazole given within 60 minutes prior to surgery and should be discontinued maximum 24-hour post-surgery. In case of allergy to the Cephalosporin, Penicillin families or Metronidazole, other IV antibiotic may be used. Pre-operation prophylactic oral antibiotic is not allowed. Mechanical bowel preparation will be at the discretion of the PI per each site's SOP. 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 all randomized subjects in these sites (sites from Czech Republic will not participate in the PK sub-study). About [redacted] series of [redacted] samples per set will be analyzed. About [redacted] series will be analyzed from subjects receiving 2 vials, about [redacted] series will be analyzed from subjects receiving 3 vials.

The occurrence of any Adverse Events (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 an overall of 60 days for safety.

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

The study will enrol a minimum of 950 subjects, with 475 subjects allocated to each treatment group.

The final number of subjects will be determined following an unblinded sample size re-estimation: when about 750 subjects will complete their 30 days (1 month) follow-up 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** of the following criteria:

1. Subjects undergoing an elective colorectal surgery involving resection, with or without a stoma formation, that includes at least 1 abdominal incision that is > 10cm (target incision).
2. Subjects are preoperative hemodynamically stable (BP≤180/110 and ≥90/60 mmHg, and HR≤100 and ≥60 bpm, and temperature ≤38.5^{0}C and ≥35.5^{0}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 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 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 (ex. toxic colitis, ileus/sub-ileus, megacolon, diverticulitis, volvulus, ect.)

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 abdominal surgery, including concomitant repair of ventral hernia. Salpingo-oophorectomy and cholecystectomy are allowed.
5. Subjects who received any anti-cancer treatment within the last 4 weeks of surgery.
6. Subjects who received radiation for colorectal cancer to the abdomen area, prior to the planned abdominal surgery.
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, other than resectable non-metastatic colorectal cancer, that is the reason for the index surgery, or carcinoma in situ (or other cancer "in situ = Stage 0"), or squamous cell carcinoma of the skin or basal cell carcinoma of the skin; or a malignancy that has not been in complete clinical remission and without maintenance chemo or immunotherapy for at least 3 years.
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 age 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.
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 ovulation[1] (oral, intravaginal, transdermal)
- Progestogen-only hormonal contraception associated with inhibition of ovulation[1] (oral, injectable, implantable[2])
- Intrauterine device (IUD)[2]
- Intrauterine hormone-releasing system (IUS)[2]
- Bilateral tubal occlusion[2]
- Vasectomised partner [2,3]
- Sexual abstinence[4]

[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.
- 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 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.

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 confirmation that the subject fulfils all inclusion criteria and none of the exclusion criteria are met, he/she will be randomized through an interactive web randomization system (IWRS) integrated with the eCRF, based on subject's information collected during the screening period and details of the planned index surgery.

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 type of SoC prophylactic antibiotic regimens (prophylactic IV antibiotic with mechanical bowel preparation or prophylactic IV antibiotic without mechanical bowel preparation) and by region (US or Europe + Israel).
Instructions and training for randomization and stratification process will be provided during the 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 re-intervention procedure (OR staff), will be trained not to disclose the treatment arm to the blinded Investigator, or other staff members, 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 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.

All blinded /unblinded activities are described in each sites' blinding plan.

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 disclosure 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.

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 within 30 days post index surgery, will be analysed as treatment failure. The blinded and independent adjudication committee review will include all index surgery incisions, regardless of any re-intervention at the target site (i.e. re-opening of the surgery incision, used for the original index surgery), for the determination of infection status.
SSI is composed of Deep SSI and/or Superficial SSI.

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

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 2020, 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 criterion for superficial incisional SSI. Conversely, an incision that is draining or that has organisms identified by culture or non-culture based testing is not considered a cellulitis.*
- *A stitch abscess alone (minimal inflammation and discharge confined to the points of suture penetration).*
- *A localized stab wound or pin site infection is not considered an SSI; 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 spontaneously dehisces, or is deliberately opened or aspirated by a physician and organism is 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 infection suspicion through eCRF page	Within 24h of awareness
Photograph of the surgical wound in case of suspected infection	During incision assessment

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.

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 by blinded Investigators (part of a Surgical Site Assessment questionnaire). 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.

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 should be followed up until resolution or permanent outcome.
Details on AE detecting, documenting, and reporting can be found in section 10.

The study sponsor's medical representative will review and assess SAEs and AEs suspected to be related to investigational medicinal product.
The sponsor will adjudicate if a safety report meet 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 Protocol Time and Events Schedule.
If any local laboratory assessments are performed during the course of the trial and they result in a change in subject management (for example due to a 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 the 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.

Clinical Chemistry Parameters
Albumin
Alkaline Phosphatase
Alanine Transaminase (ALT)
Aspartate Aminotransferase (AST)
Blood Urea Nitrogen (BUN)
Calcium
Chloride
Creatinine
C-Reactive Protein (CRP)
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

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)
Urinalysis – to be done at Screening visit and as per Investigator discretion
pH Specific Gravity Protein Glucose Bilirubin Ketones Nitrites Leukocytes Erythrocytes

Pregnancy test (β-HCG) by serum or urine dipstick at screening only, will be performed in female subjects of child-bearing potential only.

Any abnormal laboratory finding compared to baseline, assessed by the Investigator as clinically significant, should be recorded as an AEs in the relevant eCRF form.

9.1.13 **Bacteriological Testing - (Should be Taken in Any Case of Suspected Infection <u>Before Starting Antibiotic Treatment</u>)**

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

Bacteriological tests from the wound will be taken if there is a wound discharge.

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 as an AE in the eCRF.

9.1.14 Physical Examination

The Investigator will perform a complete physical examination of the major body systems and will assess whether the subject experiences Doxycycline reactions or other allergies.
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 and the time of measurements will be recorded in the eCRF.
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 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

according to CDC criteria and ASEPSIS scoring, as well as modified Vancouver questionnaire[1] 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, using their cellular phone per instructions provided as a separate manual. The photo(s) should 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 un- willing 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.

9.1.20 Pharmacokinetics

Doxycycline pharmacokinetic sampling will be collected in selected sites from all randomized subjects in these sites. About [redacted] series of [redacted] samples per set will be analyzed (sites from Czech Republic will not participate in the PK sub-study). About [redacted] series will be analyzed from subjects receiving 2 vials, about [redacted] series will be analyzed from subjects receiving 3 vials. Pharmacokinetic samples of [redacted] blood will be collected at each PK time point, prepared and shipped to the central laboratory.

[1] Modified Vancouver questionnaires will be completed following wound closure

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Visit / Day	Time after surgery	Comments
Screening (Visit 1)	Pre-application of D-PLEX (can be done on Day 0 (Visit 2) before Surgery)	Only if Screening visit performed a day before surgery
Surgery - Day 0 (Visit 2)	Pre-application of D-PLEX (if not done during screening) After D-PLEX Application: [redacted]	Subject hospitalized
Day 1 (Visit 3)	[redacted]	Subject hospitalized
Day 2	[redacted]	Only if subject still hospitalized
Day 3	[redacted]	Only if subject still hospitalized
Day 5 (Visit 4)	[redacted]	
Day 14 (Visit 5)	[redacted]	
Month 1 (Visit 6)	[redacted]	
Month 2 (Visit 7)	[redacted]	

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

- 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 allergy questionnaire.
- Record concomitant medications.
- Measure height, weight and vital signs (body temperature, heart rate & blood pressure).
- Perform physical examination.
- Hematology and Chemistry for Central Laboratory *.
- Coagulation and Urinalysis in local lab (tests done at the local lab 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) - local serum test or urine dipstick should be performed not more than 1 day before surgery.
- 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

**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 mechanical bowel preparation in the preceding several days. 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 or 2nd generation of Cephalosporin family and Metronidazole given within 60 minutes before surgery and should be discontinued maximum 24-hour post-surgery. In case of allergy to the Cephalosporin/Penicillin families or Metronidazole, other IV antibiotic may be used. Pre-operation prophylactic oral antibiotic is not allowed. Mechanical bowel preparation will be at the discretion of the PI per each site's SOP.

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 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 & 6h) – 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-surgery. These visits are usually performed after this type of surgery.

The additional follow-up visit (visit 6) will take place 30 days post-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 (only if wound is not dressed).
  - Modified Vancouver questionnaires 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
- Urinalysis will be performed only at the discretion of the Investigator.
- Bacteriology cultures will be taken if there is wound discharge.

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.

- The date and reason for the unscheduled visit will be recorded.
- Assessment of surgical site,
- Vital signs,
- AEs
- 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.

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)	Visit 6 Day 30 (+ 7 days)	Visit 7 Termination Day 60 (± 7 days)
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 (serum or urine dipstick)[9]	X						
Assessment of Surgical Site[6]			X	X	X[5]	X[5]	X[5]
Blood Tests (hematology, chemistry[3])	X[2]		X	X	X	X	X
Urinalysis [2,4]	X						
Bacteriology test[7]			X[7]	X[7]	X[7]	X[7]	X[7]
Doxycycline PK Sampling[8]		X	X	X	X	X	X
Adverse Events		X	X	X	X[5]	X[5]	X[5]
Concomitant Medications	X	X	X	X	X	X	X
D-PLEX Administration		X					

Notes:

1. Confirmation of Eligibility.
2. May be performed at visit 2 (before surgery).
3. Blood **chemistry and hematology**: for whole list of tests please refer section 9.1.11. **Coagulation** tests (PT, INR, PTT) only at Screening.
4. **Urinalysis** will be done at screening visit and at the discretion of the Investigator during the other study visits.
5. In case the subject is unable to attend a visit, , phone visit should be performed with subject/his community physician/ a first degree relative (as per Sponsor's designated form) to obtain information related to subject's safety and wound assessment. Blood tests and vital signs can be collected in the local clinic/laboratory and the results should be send (blinded with subject study number only) to the Sponsor study manager.
6. Surgical site assessment will be performed only if wound is not dressing
7. Bacteriology test will be performed in case of suspected infection and wound discharge
8. Blood samples for PK will be collected from about [redacted] subjects at selected sites at the following time-points: [redacted].
9. Pregnancy test should be performed within 1 day before surgery.

9.3 DEFINITION OF END OF TRIAL

Total duration of the study is expected to be approximately 12 months from first subject enrolled until last subject completed. 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.

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 [redacted] which is equivalent to [redacted] Doxycycline free base. Doxycycline is being constantly released for approximately 4 weeks 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:

10.1.3.1 **Product Preparation - Step 1:**

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

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
10 - ≤20	2
>20	3

1. 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 contents of vial is sterile.**
2. Make sure that the integrity of the package and the vial is not damaged. **If it is damaged - do not use it.**
3. Put a pair of **new, sterile** gloves.
4. 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).
5. Wait until alcohol evaporate.
6. Shake the vial well.
7. Ensure a mixing bowl is ready
8. Remove the vial's cap by flipping the cap up, and turning it counter clockwise, according to the arrows stamped on the cap.
9. Open the vial slowly and do so close to a sterile mixing bowl using standard aseptic techniques.
10. 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.
11. In case not the entire content of the vial was not 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.

10.1.3.1 Product Preparation - Step 2:

To be conducted by a sterile nurse / surgeon:

12. Slowly hydrate the D-PLEX powder in the bowl with the following volume 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.
13. Do not over hydrate.
14. Mix the D-PLEX powder, with sterile saline using a sterile spatula, for approximately 1 minute, reaching a uniform paste appearance.
15. 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.

10.1.3.2 Product Administration - Step 3:

***This step should be done by the operating surgeon*.**

16. The paste is to be applied by the surgeon, after wearing a new set of sterile gloves.
17. **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.
18. 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).
19. 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 used in this trial will be chosen according to the following scheme:

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

D-PLEX will be applied directly within the surgical site immediately after hydration and mixing, 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 overdose. Per surgeon decision, the administered D-PLEX can be removed by re-exploring the surgical incision and washing out the D-PLEX.
All overdoses should be recorded and notified to the Sponsor.
If an SAE is associated with the overdose, the PI should ensure the overdose if 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 as per protocol.
- Additional antibiotics (excluding Doxycycline) **for the treatment** of medical history condition or AE.

10.2.2 Medication(s) Prohibited During the Trial

- Additional prophylactic antibiotics (including antimicrobial sutures).
- High steroids dose (more than 80mg per day).
- 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]. 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.
Information on the Product Handling (storage & preparation) can be found in section 0 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 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

  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.

10.7 ASSESSMENT OF ADVERSE EVENT

AEs and SAEs will be reported in accordance to time period and frequency for detecting AEs section 10.9

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.

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 Sequalae	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.

10.8 **DOCUMENTATION AND REPORTING OF SAE AND SSI**

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

SAEs 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 queries raised by the Sponsor are responded as soon as possible.

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

1. SAE Term – if diagnosis was provided, the SAE term should be the diagnosis, otherwise, symptoms should be reported as separated AE/SAEs.
2. SAE onset date - report the start date the event became SAE.
3. SAE 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
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 (e.g. Treatment, Other Action)
9. Outcome (Ongoing, Resolved with Sequelae, Resolved, Death)
10. SAE 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 S/AE. or

AEs 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 and SSIs will be reported to Sponsor as indicated in the table below:

	Initial Reports		Follow-up Reports	
Type of Event	**Time Frame from awareness**	**Documents**	**Time Frame from awareness of New Information**	**Documents**
SAEs	24 hours	eCRF	24 hours	eCRF
AEs which were assessed by the PI as 'not related' to the IMP	5 days	eCRF	5 days	eCRF
AEs which were assessed by the PI as 'related' to the IMP	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 patient 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.

10.10 REPORTING OF SUSARs (SUSPECTED UNEXPECTED SERIOUS ADVERSE REACTION)

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 regulationsInvestigator will forward SUSARs that occurred at his center to the local EC/IRB in a timely manner, per local regulation. All other SUSARs will be forwarded to EC/IRB periodically (at least every 6 months), based on local regulation.

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 should be followed up until resolution or permanent outcome.

For SAE - 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 hour 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 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-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 study will enrol a minimum of 950 subjects, with 475 subjects allocated to each treatment group. The sample size calculation was based on powering the study with respect to the primary efficacy endpoint. It was assumed that the SSI rate in the SOC arm would be 16% and that D-PLEX +SOC will have effect of 50% on this rate (halving infection rate) relative to SOC alone, thus 8% infection rate in D-PLEX+SOC arm. Assuming rate equal to [redacted] for both arms with regard 30-day treatment failure due to all-cause mortality or re-intervention in the target incision, due to suspected SSI or due to poor wound healing, including wound dehiscence, the assumed treatment failure rates were [redacted] and [redacted], for SOC and DPLEX+SOC, respectively. A sample of 882 total subjects provides 90% power to detect the assumed difference in treatment failure rate using chi square test, and to account for the planned interim analysis for efficacy (to be performed with a two-sided α=0.01), a conservative two-sided α=0.04 level of significance was employed. In order to account for an anticipated about 5% lost-to-follow-up rate, a minimum of 950 subjects (475/treatment arm) will be enrolled.

14.1.1 Interim analysis for stopping early for efficacy or futility, or un-blinded sample size re-estimation-

One comparative interim analysis for an early efficacy or futility stop, or sample size re-estimation will be conducted when about 750 subjects will complete 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 (950 subjects) or continue with sample size increased up to a total of 1400 subjects, only if the comparative result falls in a predefined 'Promising Zone'. The interim decision will be made by the independent DMC according to a pre-planned interim analysis plan, and comparative information will be maintained confidential within the independent statistician until the study finally unblinded. The 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.

14.2 ANALYSIS POPULATIONS

The following analysis populations will be defined for the study:

14.2.1 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. In this analysis set, treatment will be assigned based on the treatment to which subjects were randomized, regardless of which treatment they actually received.

14.2.2 Safety Analysis Set

The safety analysis set will consist of all subjects who have been randomized and treated with D-PLEX+SoC 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.

14.2.3 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 index surgery, 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. Subjects who will die during the study will not be excluded from the mITT population.

14.2.4 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, due to suspected SSI or due to poor wound healing, including wound dehiscence).

The analysis of the primary and key secondary efficacy endpoints will be performed on the ITT, mITT and PP populations, with the ITT population serving as the primary analysis 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 ITT population.

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 ITT population by treatment group and overall. Continuous variables will be displayed via summary statistics (number of non-missing observations, mean, median, standard deviation, 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 DPLEX+SOC over a period of 30 days post operation, by preventing surgical site infection (SSI), defined as superficial and/or deep infection, in the index (target) incision, compared to the SoC alone control arm.

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 event, 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 (due to suspected SSI or due to poor wound healing, including wound dehiscence, as determined by the adjudication committee) 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 or re-intervention due to any other reason than suspected SSI or due to poor wound healing, including wound dehiscence, will not be considered as a treatment failure in the primary analysis.. 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 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. A 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.

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

The interim analysis adjusted significance level will be used for this test, refer to 14.3.4 for details. 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).
- Descriptive statistics of the incidence of re-interventions at the target incision site due to other reasons than suspected SSI or due to poor wound healing, including wound dehiscence within 30 days of the index operation. Analysis where these events will also be considered as treatment failure (in addition to the primary endpoint defined above) will also be performed.

14.3.3 Secondary Efficacy Endpoint Analysis

14.3.3.1 Key secondary efficacy endpoint

The key secondary endpoints defined for the study are:

- Infection rate as measured by the proportion of subjects with at least one SSI event only, occurring within 30 days post abdominal surgery and determined by a blinded and independent adjudication committee.
- 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 who reached a score of ASEPSIS>20 within 30 days post abdominal index surgery will be presented by group. Method of analysis of this endpoint will be as described for the 1st key secondary endpoint above. This endpoint will be formally tested only if the 1st key secondary endpoint is significant, using two-sided 5% significance level.

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 efficacy endpoints are 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, the key secondary endpoints will be interpreted inferentially only if a statistically significant treatment effect (p-value ≤0.05) is detected in the primary endpoint. 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 750 subjects in total will be enrolled and have the chance to complete 1-month visit, and an alpha level of 1% regardless of the exact number of subjects included in the analysis will be used. For strict control of the overall type-1 error, the alpha for the final analysis (in case the study does not stop at interim) will be calculated based on the actual information time of the interim analysis.

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).

14.3.5 Subgroup analysis

The primary and key secondary endpoints will be analysed by incision length with the following categories: ≤20 cm, >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, due to suspected SSI or due to poor wound healing, including wound dehiscence, 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, due to suspected SSI or due to poor wound healing, including wound dehiscence, will be imputed as treatment failure.

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
When using mITT and PP populations: no imputation will be performed (i.e., a complete case analysis will be used).
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 formal analysis to stop the study early for efficacy or safety is planned for this study. The interim analysis will include a group-sequential design stopping rule for efficacy or futility, combined with comparative sample size re-estimation (please refer to section 14.1.1).

14.4 NAME OF COMMITTEES INVOLVED IN TRIAL

14.4.1 DATA MONITORING COMMITTE (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.

With regard to the interim analysis, the interim decision will be made by the independent DMC according to a pre-planned interim analysis plan, and comparative information will be maintained confidential within the independent statistician until the study finally unblinded.

14.4.2 ENDPOINTS ADJUDICATION COMMITTEE (EPAC)

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". In addition, the committee will review all death cases to verify if they may be related to abdominal wall infection, IP or other cause. It is within their scope to make an independent decision whether a subject has met the primary endpoint or to assess, in case of a death, for its relationship to infection or to the study drug.
Vote followed by decision will be made based on an agreement of at least 2 out of 3.
Committee's adjudication blinded review will be forwarded to the Sponsor.
In case of dispute between Investigator assessment of infection and the committee's assessment, 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 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, 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 Investigatory/ 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 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

23 REFERENCES

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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]

	Proportion of wound affected, %					
Points for:	**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

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

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 K_2 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 poly-propylene 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 or below -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 (e.g. Set 'A' is sent first, followed by Set 'B', dispatched 24h to 72h later).
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 -20°C prior to shipment to: ICON Laboratories, Inc.

Back-up Sample Shipment

Back-up samples are to be shipped once ICON Laboratory confirms the primary samples have been received. 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.

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^{0}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 **drainage (exudates)** 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)		

Organisms identified by microbiologic testing method		
Superficial incision that is **deliberately opened** (i.e. removal of pins to better examine the incision)		
A deep incision that **spontaneously dehisces**, or is **deliberately opened** 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)		
Diagnosis of a Superficial incisional SSI **by the assessor**		

Final Assessor Decision

	Yes	No
Superficial Surgical Site Infection		
Deep Surgical Site Infection		

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 after-load 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
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)	

Medical Condition	Score
None	0
Mild	+1
Moderate to severe	+3
Diabetes mellitus	
None or diet-controlled	0
Uncomplicated	+1
End-organ damage	+2
Hemiplegia	
No	0
Yes	+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	0
Yes	+2
Solid tumor	
None	0
Localized	+2
Metastatic	+6
Leukemia	
No	0
Yes	+2
Lymphoma	
No	0
Yes	+2
AIDS	
No	0
Yes	+6
Is this a COVID-19 patient? For research purposes only; answer does NOT impact results. Confirmed positive Suspected Unlikely Confirmed negative	

Summary score	

APPENDIX 6: DOCUMENT CHANGE HISTORY

Revision	Changes	Reason for changes
Version 01	NA	First Issue
Version 02	Title, Summary, Section 8.1, 9.1.4, 9.1.5, 9.1.6 - Change in the study blindness level and clarification	Level of blindness was changed from single to double due to FDA and EMA requests and changes in the blindness procedure.
	Summary, Inclusion criteria, Sections 8.1, 8.4 - Change of eligible surgery types	Type of surgeries eligible for the study was changed according to updated data of infection rate after different type of abdominal surgeries.
	Summary, Inclusion criteria, Sections 8.4, 8.5– clarification and re-wording of Inclusion/Exclusion criteria	Inclusion/Exclusion criteria were slightly clarified and re-worded to be clear to the study site team
	Summary, Section 8.1, 9.1.3, 9.2.2, 10.2.1 - SoC was more detailed	SoC was more clarified due to EMA request.
	Summary, Sections 8.1, 9.1.3, 14 - Change of Patient stratification plan	Patient stratification plan was updated according to updated type of surgery and type of SoC prophylactic antibiotic regimens.
	Sections 9.1.16, 9.2 (includes all subsections) – ECG test was cancelled	ECG test was cancelled as this procedure is standard of practice before surgery and not needed to be duplicated in the study.
	Summary, Sections 8.3, 14.1 - Change of Sample size	Sample size was changed due to updated estimation of infection rate in US and Europe
	Summary, Sections 8.1 - Change of laparoscopic surgeries limitation	Part of laparoscopic surgeries in the study was decreased due to updated estimation of infection rate in US and Europe
	Summary, Sections 8.3, 14 - Changes of statistical plan	Blinded analysis was combined with unblinded Interim Analysis
	Summary, Sections 7.2, 14.3.3 -	Key secondary endpoints were changed due

Revision	Changes	Reason for changes
	Change of key secondary and additional endpoints	to updated review of Phase 2 results and discussions with KOLs and statisticians
	Section 14 – Statistical analysis were changed	Statistical analyses were changed according to the change of key secondary and additional endpoints
	Section 5.3 – update of Phase II study results	Phase II study results were updated according to Final Statistical report
Version 03	Summary, Sections 8.3, 14.1 - Change of Sample size	Sample size was changed due to updated estimation of the infection rate in the US and Europe
	Summary, Section 9.1.20 – PK study was added	PK study was added following HPRA request
	Summary, Sections 8.3, 14 - Changes of statistical plan	Statistical plan was updated following change in analysis of the Primary Endpoint
	Summary, Section 8.1, 9.1.3, 9.2.2, 10.2.1 – change of prophylactic antibiotic treatment	Prophylactic pre-operative oral antibiotic was removed and will not be allowed during this study
	Summary, Section 5.6, 10.1.4 - change of re-application during re-intervention surgery	Re-application of D-PLEX during re-intervention will not be allowed following discussion with the FDA and HPRA
	Section 9.1.11 – change of chemistry tests	Creatinine Phosphokinase (CPK) test was added as was omitted in this section by mistake
	Table 1 (Study assessments flowchart) was updated	Table 1 (Study assessments flowchart) was updated according to the protocol corrections
	Summary, Section 8.1 – change of incision length	Allowed incision length was changed following discussions with KOLs
	Summary, Sections 7.2, 14.3.3 - Change of primary, key secondary and additional endpoints	Primary, key secondary and additional endpoints were changed due to discussions with the FDA, HPRA and statisticians
	Section 14 – Statistical analysis were changed	The statistical analyses were changed according to the changes of study design (changes in sample size, primary and key

Revision	Changes	Reason for changes
		secondary endpoints, stratification plan), following the FDA request
	Section Summary, 5.1.1.4, 9.1.20, 10.1.4 – Change in number of vials should be used	Number of vials to be used was changed due to change of eligible surgeries (incision length > 10cm)
	Summary, Section 9.1.6 – CDC SSI criteria were updated	CDC SSI criteria were updated according to updated CDC/NHSN Patient Safety Component Manual (January 2020, chapter 9).
	Appendixes – Addition of Surgical Site Assessment Questionnaire	A Surgical Site Assessment Questionnaire was added as appendix
	Appendixes – moving of document history of the previous versions to appendix	Document history of the previous versions was moved to appendix to better protocol format
Version 04	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.
	Summary, Sections 8.1, 9.1.20, 9.2 – clarification of PK collection process	PK collection process was updated.

Revision	Changes	Reason for changes
	Section 9.1.16 – Medical History and Concomitant Medication collection was updated	Medical History and Concomitant Medication collection were 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 10.1.4 – Product dose table was corrected	Typo mistake was corrected in the Product dose table.
	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
	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 clarified 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 05	Summary, Sections 8.1, 9.1.20 – the PK testing will not be	As per Sponsor decision

Revision	Changes	Reason for changes
	performed in Czech Republic.	
	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) 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

Revision	Changes	Reason for changes
	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 timelines were updated	Clarification
	Section 10.10 and 10.11– SUSAR and DSUR reporting process were re-phrased	Clarification

Document Signatures

Participant Name	Participant Type	Action	Task Performed On
Olga Belotserkovsky (Clinical Development Manager)	Approver	Send to QA Approval	2022-02-21 17:23
Lital Weinfeld Bergman (Clinical Operations Manager)	Approver	Send to QA Approval	2022-02-21 18:11
Dalit Hazan (VP R&D and RA)	Approver	Send to QA Approval	2022-02-21 18:30
Noam Emanuel (CSO)	Approver	Send to QA Approval	2022-02-22 09:45
Maria Rubin (VP Quality)	QA Approver	Approve Document	2022-02-22 10:02