Study Number:	**Study D-PLEX 310**
Full Title of Trial	Phase II, Prospective, Multicentre, Randomized, Controlled, Two arm, Single Blind, Study to assess Safety and efficacy of D-PLEX Administered Concomitantly with the Standard of Care (SOC), compared to 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 04**
Protocol date	**27 Nov 2018**
Sponsor	PolyPid Ltd, 18 HaSivim Street, Petach Tikva, Israel, 4917002 [redacted]
Sponsor Protocol Number	CL-0013, Study D-PLEX 310
Phase of Trial	Phase II
Site(s)	[redacted]centers in Israel will participate in this study.
Sponsor Representative	[redacted] VP Clinical PolyPid Ltd. 18 HaSivim Street, Petach Tikva, Israel, 4917002. [redacted]

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SPONSOR SIGNATURE PAGE

Protocol #:	**Study D-PLEX 310**
Protocol Title:	Phase II, Prospective, Multicentre, Randomized, Controlled, Two arm, Single Blind, Study to assess Safety and Efficacy of D-PLEX Administered Concomitantly with the Standard of Care (SOC), compared to SOC treated control arm, in prevention of post abdominal surgery incisional infection.
Protocol Version:	**04**
Protocol Date:	**27 Nov 2018**
Sponsor:	PolyPid Ltd. 18 Hasivim Street, Petach Tikva, 4917002, Israel [redacted]
Sponsor Representative Name:	[redacted]
Title:	VP clinical
Signature:	[redacted]
Date:	27 nov 2018

INVESTIGATOR SIGNATURE

Protocol #:	**Study D-PLEX 310**
Protocol Title:	Phase II, Prospective, Multicentre, Randomized, Controlled, Two arm, Single Blind, Study to assess Safety and Efficacy of D-PLEX Administered Concomitantly with the Standard of Care (SOC), compared to SOC treated control arm, in prevention of post abdominal surgery incisional infection.
Protocol Version:	**04**
Protocol Date:	**27 Nov 2018**

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

_____ _____

Principal Investigator (signature) *Date*

DOCUMENT HISTORY

Revision	Date reviewed/revised	Changes
Version 04	27 Nov 2018	• Correcting typo related to blood tests and urinalysis time frame at screening • Amending non-clinical information
Version 03	14 Nov 2018	• Change of number of participating centers and recruited patients • Addition of a secondary endpoint • Adjustment corrections to table 1 • Changes to entry criteria • Amendment of the statistical plan • Allow for blood test for screening to be done up to 21d prior to surgery • Add a +1d window for V4 (D5) – if occurs on a Saturday/holiday • Removal of bodily systems description in physical examination • Clarify the administration instruction for D-PLEX • Removal of the option "LTFU" from AE outcome • Amend PK preparation instructions
Version 02	01 April 2018	• Updates of pre-clinical and clinical data • Updates to the number of patients and sites • Eligibility criteria updates • Updating and emphasizing the index surgery and incision • Determining SOC pre-operative treatment

		• Insertion of operational Instruction for Use (IFU) • Adding a statistical analysis plan • Corrections of inconsistencies throughout the protocol
Version 01	NA	First Issue

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

Revision	Changes	Reason for changes
Version 04	Sections: Change History, Document Change History, sections 8.2.1 & 8.3	Following a change in version 03, allowing 21 days as a time frame for blood tests and urinalysis prior to surgery to be used for eligibility, this version corrected typo that were mistakenly not changed in the previous version.
	Section: 2.2	Updating non-clinical info based on new available safety data.
Version 03	Sections: cover, summary, Change of number of participating centers and recruited patients	Adding [redacted] centers and an option to increase number of recruited patients up to [redacted] following assessment of the primary endpoint after [redacted] recruited patients.
	Sections: summary and 4.2 Addition of a secondary endpoint	2nd endpoint of number of re-admissions due to SSI was omitted in the previous version.
	Sections: 8.3 Adjustment & corrections to table 1	• Adding the test of rectal swan at screening and D30 to the procedures table (section 8.3) • Add clarification for ECG allowed window • Change window for blood tests from – 1 week to – 21 days
	Section: Summary and 6	

	Clarification to Exclusion # 2 Add inclusion criteria for survival expectancy after randomization	• Addition of an inclusion criteria re survival expectancy post randomization • Adjustment to exclusion # 2 for greater clarity.
	Sections: Summary and 14.1 Amendment to the statistical plan	Addition of a blinded re-assessment after [redacted] recruited patients for evaluating the infection rate precision assumptions
	Sections: 8.1.9, 8.2.1 and 8.3 Allow for blood tests and urinalysis for screening to be done within 90d prior to surgery	To align with the sites standard practice re tests prior to surgery.
	Section 8.3 Add a +1d window for V4 (D5) – if occurs on a Saturday/holiday	To allow for tests to be done and avoid deviation.
	Sections: 8.1.11 Removal of bodily systems description in physical examination	To align with the sites standard practice re tests prior to surgery.
	Sections: Summary, 2.1.1.4 and 9.1.4 Clarify the administration instruction for D-PLEX	Greater clarity
	Section 10.2.3 Removal of the option "LTFU" from AE outcome	Was mistakenly left at last protocol update.
	Section: Appendix 1 Amending time for storage prior to centrifuge	Following new stability test, amending storage length from 12h to 18h for PK.
Version 02	Sections: opening pages, summary & throughout the protocol Adding and re-wording of <u>study objectives</u>	To better describe the goal of the study

	Section: opening page, sponsor and investigator signature pages Re-wording of study title	To better describe the study design
	Sections: summary & 6 Correction to number of study participants and centers	Changes to the number of required subjects and sites
	Sections: Summary, 5.1, 6.1, 8.2.1 & 10.1.3 Adjustment of subject population description	Adding "elective", enhancing the index surgery as "colorectal" and leaving out details to the study inclusion criteria.
	Sections: 2.1.2, 2.1.3, 2.2 & 2.3 Re-written the pre-clinical and clinical sections	To include new data from pre-clinical and clinical studies
	Sections: NA Deleted data on BonyPid product.	Focusing on pre-clinical and clinical data from studies with D-PLEX
	Section 2.5 Correction of mitigation "g" Re-wording of mitigation "k"	Clarifying the instruction of re-allocation of an un-used study product. Changing "may" to "should" and amending instructions.
	Sections: summary, 4 & 8.1.5 - endpoints	4.1 - Additional clarification re definition of superficial SSI, as per CDC guidelines - Adding death within 30 days post-operation as treatment failure - Adding requirement for a blinded assessor for the surgical wound 4.2 – adding secondary endpoints. 4.3 & 8.1.5 – highlighting the role of the adjudication committee as a blinded and an independent one.
	Sections: Summary & 5 Study design amendments to include different changes	• Emphasizing requirement for at least 1 X 5cm incision • Shortening the follow-up period to 60 days

		• Describing the minimal surgery requirements
	Sections: Summary, 5.1 & 8.2.2 Determining the pre-operative standard of care treatment	To align the pre-operation prophylactic treatment across all sites, as per standardizes MOH's and international guidelines
	Sections: Summary & 6 – entry criteria • Cancel the upper limit to patients age for study inclusion • Include "able and willing" subjects • Allow patients with active GI (only) infection to be included • Pending sponsor's approval, additional concomitant procedures, for the benefit of the subject, may be allowed during the index surgery • Restrict chemotherapy and radiation treatments • Removal of restriction for immunocompromised subjects	• Following feasibility at new sites and an FDA comment • To also include patients with chronic GI illnesses • Following comments from the study PIs, in order not to exclude all patient requiring of a secondary procedure. • Due to inclusion of oncology patients. • To allow patients with immuno-gastric diseases.
	Section 8 – study procedures 8.1.2 & 8.1.3 – Merge the definitions of randomization/enrollment 8.1.4 - Adding requirement for a blinded assessor for the incisional wound 8.1.4.1 - Adding description for emergency un-blinding 8.1.16 – Adding infection assessment questionnaires 8.1.9 – Adding a test (rectal swab)	As per FDA comment, the 2 should be the same. To maintain blind as much as possible (in a single blinded study) Following requirement for a blinded assessor Adding a scoring questionnaire (ASEPSIS) to the visual assessment of wound infection. Following advice of an infectious disease's expert – to ensure resistance is not created.

	8.1.10 – Adding a central lab	All suspected incisional-site-infection tests will be processed in a central lab to standardize process between all participating patients.
	8.1.14 – concomitant medications	Adjusting instruction following deletion of entry criteria # 13
	8.1.17 - Pharmacokinetics	Amending the time point for PK collection and dividing the samples between the 3 doses planned for the study. Adding guidance for case of re-application
	Section 8.2.3, 8.2.5 & table 1 – <u>study visits</u>	• Removing visit No. 8 & amending visit 7 to be a termination visit • Adding a rectal swab test to Screening & V7 as per secondary endpoint. • Adding infection assessment questionnaires to the visual check at all study visits.
	Section 8.3 – Table 1 (bullets)	Adding description of the PK timelines and guidance to data collection in case of a patient no-show.
	Section 9 – <u>study treatment</u> 9.1 – study treatment D-PLEX	Inserting data (description, composition, storage and preparation, application, contra-indications, warnings & packaging) from the **I**nstruction **F**or **U**se (IFU) into the protocol and cancelling the additional leaflet of IFU.
	9.2 – concomitant medications	Adjustment as per revised entry criteria and pre-operation SOC.
	9.4 – drug accountability	Deletion of guide for destruction at site as not applicable for this study.
	Section 10.2.2 & 10.2.3 – <u>Adverse Events</u> Change in categories for causality and outcome	Amending options for causality and outcome.

	Sections summary & 14 – statistical analysis	A statistical analysis plan was added.
	GENERAL	Rephrasing, deletion of duplicates, correction of mis-spelled words or not clear enough instructions.

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LIST OF ABBREVIATIONS

AE	Adverse Event
ACS	Acute Coronary Syndrome
AR	Adverse Reaction
ASC/AST	Active Surveillance Culture/Testing
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
ALT(SGPT)	Alanine Aminotransferase
AST(SGOT)	Aspartate transaminase
BMI	Body Mass Index
β-TCP	β- Tri Calcium Phosphate
CDC	Center for Disease Control
CKD	Chronic Kidney Disease
Cm	Centimetres
eCRF	Electronic Case Report Form
CVA	Cerebro Vascular Accident
DSSI	Deep incisional Surgical Site Infection
EC/IRB	Ethics Committee/Independent Review Board
ECG	Electrocardiogram
ESRD	End Stage Renal Failure
EU	European Union
G	Gram
GCP	Good Clinical Practice
GI	Gastrointestinal
GMP	Good Manufacturing Practice
GRAS	Generally Recognized As Safe
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IFU	Instructions for Use

IP	Investigational Product
ISO	International Organization for Standardization
IV	Intravenous
LDH	Lactate Dehydrogenase
MG	Milligram
MOH	Ministry of Health
MRSA	Methicillin-resistant Staphylococcus aureus
MSSA	Methicillin-sensitive Staphylococcus aureus
NHSN	National Healthcare Safety Network
PI	Principal Investigator
PK	Pharmacokinetics
PLGA	Poly (DL-lactide-co-glycolide)
PLEX	Polymer- Lipid Encapsulation matrix
PVD	Peripheral Vascular Disease
QA	Quality Assurance
QC	Quality Control
SAR	Serious Adverse Reaction
SAE	Serious Adverse Event
SDV	Source Document Verification
SOC	Standard of Care
SOP	Standard Operating Procedure
SSI	Surgical Site Infection
SSSI	Superficial incisional Surgical Site Infection
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment emergent adverse event
TIA	Transient Ischemic Attack
USP	United States Pharmacopeia

TRIAL PERSONNEL

Sponsor's Representatives

1. [redacted]
   VP Clinical
   PolyPid Ltd.
   18 HaSivim Street, Petach Tikva. Israel 4917002
   [redacted]

1 SUMMARY

Study Number:	Study D-PLEX-310

Study Title:	Phase II, Prospective, Multicentre, Randomized, Controlled, Two arm, Single Blind, Study to assess Safety and Efficacy 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 II
Objectives:	The primary objectives are as follows: • To assess the anti-infective efficacy of D-PLEX over a period of 30 days post operation, by preventing surgical site infection (SSI), defined as superficial and deep infection, in the target incision(s), compared to the SOC treated control arm. • To assess the safety of D-PLEX
Number of Centers:	[redacted] centers in Israel will participate in this study.
Subject Population:	Male and female, 18 years old and above at screening, undergoing elective abdominal colorectal surgery.
Number of Subjects:	About [redacted] subjects: A blinded non-comparative sample size re-assessment to evaluate the infection rate precision assumptions will be conducted: after about [redacted] subjects, and about [redacted] subjects, have been assessed for primary efficacy endpoint. The maximal sample size allowed to upsize is about [redacted] subjects.
Test Product:	D-PLEX is a new formulation of extended release of Doxycycline. Each [redacted] D-PLEX is supplied as a sterile powder to be reconstituted to paste in the operating room, using standard aseptic techniques and is intended for single administration. The non-active components of the extended release antibiotic formulation are β Tri-Calcium Phosphate (β-TCP), poly (DL-lactide-co-glycolide acid) or PLGA, and a lipid matrix. 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 (SOC):	The SOC prophylactic antibiotic treatment which will be used in this study is based on the MOH's (Berlowitz 2001) and international (Ongom & Kijjambu 2013, Bratzler 2013) guidelines. It will be consistence and standardized for all sites in the clinical study and is composed of antibiotic treatment from the 1st or 2nd generation of Cephalosporine family, plus Metronidazole. Bowel preparation (mechanical only) and post-surgery treatment will be at the discretion of the PI as well as the SOC per each site's SOP, as applicable.
Dose Administration:	D-PLEX dose is individualized, pending length of the surgical incision; up to a total max of [redacted]) may be administered in a single application.
Duration of Dosing:	D-PLEX is administered as a single application. The active material (Doxycycline) is continuously released for approximately 3-4 weeks.
Study Design:	This is a phase II, prospective, multicenter, randomized, controlled, two arm, single blind study. The study population includes male and female, 18 years old and above at screening, undergoing elective abdominal colon surgery involving resection and including at least 1 incision that is ≥ 5cm. Subjects who meet the inclusion criteria and none of the exclusion criteria and provide signed informed consent 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 blinded to the study arm. 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. For subjects randomized to the control arm, the surgical treatment will be as per SOC as outline in this protocol. For both arms the pre-operative prophylactic antibiotic care will be consistence and standardized for all sites in the clinical study. Post-operative care will be performed per site SOC. Post-operative resumption of activity is at the discretion of investigator based on subject medical condition.

	Doxycycline pharmacokinetic sampling will be collected from three subsets administered with different entire dose of D-PLEX [redacted]) in at least [redacted] patients (i.e., [redacted] patients per subset, pending subset recruitment). The occurrence of any adverse events (AE/SAE) will be recorded throughout the study. The planned clinical study will evaluate the safety and efficacy of the controlled release antibiotic (Doxycycline) in the prevention of post abdominal surgery incisional infection over a period of 30 days for the primary outcomes. All patients 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 abdominal incisional infection event, as determined by a blinded and independent adjudication committee, within 30 days post abdominal surgery. [abdominal incisional infection is composed of Deep Incisional Surgical Site Infection (DSSI) and Superficial Incisional Surgical Site Infection (SSSI)]. Mortality from any reason within 30 days post index surgery, will be analyzed as treatment failure. An independent and blinded adjudication committee will review each case suspected for SSI and will adjudicate if meets an endpoint. <u>Secondary Efficacy Endpoints:</u> <u>Key endpoints:</u> • Number of hospitalization days post colorectal surgery due to SSI • Average ASEPSIS assessment score during 30 days post-surgery. • Number of Surgical Intervention due to SSI's <u>Additional endpoints:</u> • Incidence of SSSI during 30 days post-surgery. • Incidence of DSSI during 30 days post-surgery • Mortality rate within 60 days post abdominal surgery

	• Determination of susceptibility to Doxycycline of any organisms recovered from an abdominal surgery incisional Infection Site. • Number of overall hospitalization days within 60 days post-surgery (including days of readmission due to surgical site infection). Primary hospitalization for surgery and, if occurred, readmission due to abdominal incisional surgical site infection. Hospitalization days will be counted as aggregated overall hospitalization days per subject. • Number of re-admissions due to surgical site infection. • Number of antibiotic treatment days (all routes of administration) due to abdominal incisional surgical site infection (data will be aggregated and presented both for overall days of antibiotic treatment all routes of administration and IV administration treatment days). • Time to surgical site infection post-surgery *Identification of a Surgical site infection will be based on CDC/NHSN Patient Safety Component Manual criteria (January 2018, chapter 9)* <u>Safety Evaluation:</u> The following safety parameters will be evaluated in this trial: • Adverse events, physical examinations & vital signs. • Incisional wound healing will be assessed by a blinded investigator, using a visual examination as well as wound assessment questionnaires. • Safety laboratory parameters: hematology, chemistry, urinalysis. 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 elective abdominal colon surgery involving resection and ileocolonic, ileorectal, colocolonic or colorectal anastomosis or with a stoma, who are preoperative stable hemodynamically. In a laparoscopic surgery an abdominal wall incision of at least ≥ 5 cm should be involved. 2. Male or non-pregnant female.

	3. Female of childbearing potential should have a negative serum pregnancy test prior to index procedure. Note: *All female of childbearing potential must agree to use a highly effective method of contraception (such as double barrier, oral or parenteral hormonal, intrauterine device and spermicide) consistently and correctly for the duration of the study.* 4. Subjects' age 18 years old and above at screening. 5. Subjects who sign a written informed consent. 6. Subjects who are willing and able to participate and meet all study requirements. 7. 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 scheduled for abdominal surgery which is classified as emergency. 2. Subjects with any preoperative active infection that is currently being treated with antibiotics. 3. Subjects receiving any antibiotic therapy in the past 4 weeks prior to enrolment besides prophylaxis or antibiotic for the treatment of the disease that consists the indication for surgery. 4. Patients undergoing concomitant additional procedures besides colon resection surgery, e.g. Hyper-thermic Intraperitoneal Chemotherapy, liver resection etc. Female sterilization surgery (i.e. salpingo-oophorectomy, hysterectomy etc.), involvement of a small bowel procedure or Cholecystectomy may be allowed, pending an advanced consultation and approval from the sponsor. 5. Subject received chemotherapy within the last 4 weeks of surgery, or radiation for colorectal cancer to the abdomen area, prior to the planned abdominal surgery (neo-adjuvant treatment). 6. Subjects that received oral or IV Doxycycline during the past 4 weeks prior to screening. 7. Subjects with known sensitivity to Doxycycline and/or to the tetracycline family of drugs or to the D-PLEX's excipients.

	8. Subjects with known allergies to more than 3 substances (an allergy questionnaire will be filled in during the screening process). 9. Subjects with history of allergic/hypersensitivity reaction to any substance having required hospitalization and/or treatment with intravenous steroids/intramuscular epinephrine or in the opinion of the investigator the patient is at high risk of developing severe allergic/hypersensitivity reactions. 10. Subjects with uncontrolled Asthma (GINA III-IV). 11. Subjects with End Stage Renal Disease (ESRD/ CKD stage 5). 12. Subjects with chronic urticaria. 13. Subjects diagnosed with TIA/CVA/ACS within the past 1 year prior to randomization. 14. Subjects that undergone any abdominal surgery and current planned surgery involves re-opening the scar of prior abdominal surgery. 15. Any subject with active malignancy or with malignancy that has not been in complete remission for at least 5 years. Excluding: * subjects with potentially resectable non-metastatic colorectal cancer which consists the indication for surgery. * Subjects who have had carcinoma in situ of the cervix, squamous cell carcinoma of the skin and basal cell carcinoma of the skin * Subjects with non-violent cancer that does not require treatment 4 weeks prior to, and throughout the entire study duration. 16. Subjects with other concurrent severe and/or uncontrolled medical condition that could compromise participation in the study (e.g. non-GI active infection, uncontrolled diabetes, uncontrolled hypertension, congestive heart failure, unstable angina, ventricular arrhythmias, active ischemic heart disease, uncompensated cirrhosis, active upper gastrointestinal (GI) tract ulceration). 17. Psychiatric, addictive, or any other disorder that compromises ability to provide informed consent for participation in this study. 18. Chronic alcoholic or drug abuse subjects.

	19. Pregnant or breast-feeding women or women of childbearing age who refuse or prohibited of using an effective contraceptive method of birth control (such as double barrier, oral or parenteral hormonal, intrauterine device and spermicide) throughout study participation including safety follow-up period. 20. Subjects that received any investigational drug within 30 days or 5½ half-lives of enrollment to the study (whichever is longer). 21. Subjects participating in any other interventional studies. 22. In the opinion of investigator, subject is not eligible to participate in the study and/or to comply with protocol requirements (e.g. due to a cognitive or medical condition).
Sample Size:	About [redacted] subjects with potential increase to [redacted] subjects following sample size re-assessment
Statistical Methods:	The study will enrol about [redacted] subjects, with about [redacted] subjects allocated to each treatment group. This sample size was chosen by the Sponsor to collect adequate data for evaluating the primary study objectives. This study is not powered to test formal statistical hypotheses. However, exploratory sample size calculations show that a sample of [redacted] subjects provides [redacted] power to detect an [redacted] decrease in the SSI rate ([redacted] versus [redacted]) at a two-sided α=0.10 level of significance. A blinded sample size re-assessment to evaluate the infection rate precision assumptions will be conducted after about [redacted] subjects have been assessed for primary efficacy endpoint. The maximal sample size allowed to upsize the study will not exceed about [redacted] subjects. **Primary Endpoint Analysis:** The number and proportion of subjects with at least one SSI, as determined by a blinded and independent adjudication committee, within 30 days post abdominal surgery will be tabulated per treatment group. A 95% confidence interval using exact binomial methods will be constructed for each proportion. Missing data for the primary efficacy endpoint will be imputed according to the methods described in Section 14.3.5 **Secondary endpoints & safety evaluation** analysis are detailed in sections 14.3.3 and 14.3.4

2 INTRODUCTION

2.1 BACKGROUND

2.1.1 Background information on the product

2.1.1.1 Product name

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

[redacted]g D-PLEX per vial contains [redacted] w/w Doxycycline hyclate equivalent to [redacted] ([redacted] mg) Doxycycline free base.

2.1.1.2 Drug Substance Chemical Name and Structure

Doxycycline Hyclate

The chemical name of Doxycycline Hyclate is [redacted] 1.

Figure 1: Structure of Doxycycline Hyclate

[redacted] M.W. 1025.89

2.1.1.3 Proposed Indication

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

2.1.1.4 Dosage form, route of administration, and dosing regimen (frequency and duration)

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

Each vial contains [redacted]g 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.

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 occasion prior to surgical wound closure following abdominal surgical procedures. The total dose applied at the surgical site will be determined based on surgical incision length. A maximum of three vials ([redacted]) may be administered. Therefore, the total amount of Doxycycline free base for a single surgical procedure with D-PLEX can reach up to [redacted] mg.

The individualized dose will follow the following scheme:

Surgical incision length (cm)	Max number of vials to apply*
5 – 10	1
11-20	2
>21	3

* Used D-PLEX paste will be measured and documented in the eCRF.

2.1.1.5 D-PLEX Product

D-PLEX is a biodegradable formulation of Beta-Tri Calcium Phosphate (β-TCP), Poly (DL-lactide-co-glycolide) (PLGA) and a lipid matrix. incorporating Doxycycline hyclate. D-PLEX is supplied in a glass vial ([redacted]) as a sterile powder to be reconstituted to paste and is intended for a single administration.

2.1.1.6 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 most isolates of a broad range of Gram-positive and Gram-negative bacteria. Doxycycline is effective for eradication of most of the bacteria known to cause surgical site infection.

2.1.2 Unmet medical need for prevention of post abdominal surgery incisional infection

Surgical site infection (SSI) are one of the most frequent complications in open abdominal surgeries, and they represent a significant cause of mortality and morbidity. SSIs rate has varied from approximately 5.0% to 30.0% of abdominal soft tissue surgeries, such as up to 4.0% of hysterectomies (Bratzler et al. 2013) and 30.0% (Mihaljevic et al. 2015) of colorectal surgeries and approximately 6.8% in urological procedures (Alonso-Isa et al. 2017) . Chemotherapy and other immunosuppressive medications affect the function of the immune system and as a result the SSI rate increases (Aga et al. 2015).

Approximately 10M abdominal surgeries are performed annually in US; of these 7.1M general (Gastroenterological) surgeries including 575,000 colorectal resection surgeries (Carney et al. 2017; Fingar et al. 2014), 2.9M Gynecological Surgeries and 608,000 urological surgeries.

SSIs may be classified as superficial incisional if limited to the skin and subcutaneous tissue, or deep incisional when involving the fascia and muscle. It is classified as organ space when involving an organ within the body cavity i.e. below the fascia. Such infections are usually manifested as abscess (Azoury et al. 2015; Fry 2013). Deep tissue and organ space SSIs are less frequently encountered

than superficial SSIs, but are associated with greater morbidity/mortality, more readmission rates, longer hospital stay, and increased overall hospital-associated costs when compared to superficial SSIs. Although the majority of 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 (Azoury et al. 2015).

Colorectal surgeries are performed for diseases such as colorectal cancer, ulcerative colitis, Crohn's disease, mechanical bowel obstruction and recurrent diverticulitis, often resulting in major reconstruction of the gastrointestinal tract. Trauma, or Intestinal injuries, ischemia, rectal prolapse and proctological disorders may also require large or small bowel resection (Kirchhoff et al. 2010).

Patients undergoing colorectal surgeries are at higher risk of developing abdominal wall SSIs because the colon and rectal tracts contain a large number of bacteria which become exposed during surgery. For most colon procedures, E. coli, Staphylococcus spp. (including community-associated, methicillin-resistant strain) and Bacteroides fragilis are the most likely organisms to be encountered. Other gram-negative species include Klebsiella pneumoniae and Enterobacter spp (Fry 2013, Fa-Si-Oen 2005).

Traditionally, the cancer organ (colon, uterus etc.) were removed through large abdominal incisions. In recent years, there has been a widespread shift toward the minimally invasive surgery (Lee et al. 2017). However, in 2013 open abdominal surgery is still the most common (>60%) surgical treatment for all abdominal surgeries especially for general abdominal, abdominal colon cancer, urology and obstetric surgeries (Carney et al. 2017, Lee et al. 2017). In addition, in most abdominal laparoscopic surgeries one incision of about 5 cm is still needed to remove the resected organ (Bennett-Guerrero et al, 2010).

In gynecologic surgery, SSIs generally fit into these categories, including superficial incisional cellulitis, deep incisional abscesses, and pelvic or vaginal cuff abscess formation. During a hysterectomy, the surgical site is in risk to be exposed to a unique variety of endogenous flora, including common bacteria of the skin, gastrointestinal tract and vaginal tract (Steiner and Strand 2017). Selection of prophylactic antibiotics must consider the need to cover a variety of gram-positive bacteria (i.e Staph. aureus, MRSA, Staph. Epidermitis and Entrococcus faecalis), gram-negative (e.g. E. coli and Klebsiella sp) and anaerobic organisms (i.e Clostridum) (Hemsell 1997).

The high incidence of SSI in cystectomies could be explained by the manipulation of the urinary and digestive tracts, the use of drains and catheters, and because these surgeries are usually performed in elderly patients with comorbidities. (Alonso-Isa et al. 2017). The most frequently isolated microorganisms reported in Spain from SSI in patients after undergoing laparotomy for urology indications were Enterococcus spp. (27.1%), E. coli (22.9%), S. aureus (14.6%) and P. aeruginosa (12.5%) (Alonso-Isa et al. 2017). According to a retrospective database of radical cystectomy patients from Boston, the most common pathogens in patients diagnosed with cystectomy SSI were Coagulase-negative staphylococci, E. coli and Klebsiella (Goldberg 2017).

Despite the routine use of prophylactic systemic antibiotics, the incidence of abdominal surgical-site infection does not entirely negate the risk. Therefore, the prevention of post abdominal surgery infection represents an unmet medical need.

2.1.3 Justification for the use of D-PLEX for prevention post abdominal surgery incisional infection

D-PLEX represents a novel formulation of Doxycycline which has the potential to be superior in prevention of post-surgical incisional infections following abdominal surgery over current standard of care preventive measures. Common practices that have been shown to reduce the incidence of SSIs include administering prophylactic antibiotics prior to incision, clipping rather than shaving the operative site, maintaining normal body temperature and oxygen supplementation perioperatively, and achieving adequate glycemic control (Azoury et al. 2015, Avkan-Oğuz et al. 2016, Pellegrini et al. 2017, Steiner and Strand 2017). Despite high compliance to guidelines, these interventions alone have not been proven to lower surgical site infection rates, which suggests that additional interventions are needed (Johnson et al. 2016).

While these measures have reduced the rate of infection, they have not eliminated it. 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 suitable for bacterial growth. 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 lymphatic fluid at the surgical site are disrupted. Therefore, systemic antibiotics and immune defences have impaired ability to reach the affected areas, causing them to be more susceptible to bacterial proliferation and to the development of infection. Low local prophylactic antibiotic concentrations, at the surgical site raises the risk of developing antibiotic resistant bacteria. However, raising the systemic antibiotic dose may cause systemic toxicity. In an attempt to overcome these problems, some surgeons dispense antibiotics locally before closing the incision. Such antibiotic treatment results only in a very short release duration of 1-2 hours and does not succeed in preventing the development of bacterial infection (Huiras et al. 2012, Bennett-Guerrero et al. 2010, Bennett-Guerrero et al. 2017, McHugh et al. 2011). In addition, the American Society of Health-System Pharmacists, SIS, IDSA, and SHEA have all stated that lacking sufficient evidence, the use of currently available topical antibiotics cannot be recommended (Edmiston et al. 2017). 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.

Doxycycline is effective for eradication of most of the bacteria known to cause abdominal surgery site infection.

As indicated above, D-PLEX is a new formulation of extended-release of Doxycycline for administration during surgery at the stage of closure of the surgical incision of the abdominal wall, and as an adjunct to the SOC treatment. D-PLEX releases Doxycycline immediately upon administration into the surgical site and continues to release Doxycycline for approximately 3-4 weeks following administration, which is the time period in which most of the laparotomy colon surgical site infection occur (Fry 2013).

During this period, the expected local Doxycycline concentration 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 3-4 weeks post its administration, D-PLEX is intended to be effective for prevention of post-abdominal surgery incisional infection.

2.2 NON-CLINICAL DATA

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

- Six Studies in rabbit sternal wound infection model (each with a different clinically relevant bacteria[1]) showed that D-PLEX at an applied dose comparable to 7.5 g administered to a 70 kg adult on a mg/kg basis, demonstrates reduction of infection [as reflected in the maintenance of overall health, the limited changes in hematologic parameters and the reduction in bacteria count isolated from the sternum wound (i.e. bone defect and surrounding soft tissue)] and infection-related toxicities.
- Intramuscular surgical site infection model in rat demonstrated the ability of D-PLEX to reduce S. aureus proliferation and infection (as shown in the reduced signs of inflammation).
- *In vitro* anti-microbial studies demonstrated D-PLEX ability to reduce the load of clinically relevant bacteria. In vitro anti-microbial study for extended period further demonstrated that D-PLEX is effective against Staphylococcus aureus (which is a common bacterium in human sternal and colorectal surgical wound infections) over 5 weeks period in which it is subject to release in vitro. This supports the claim that D-PLEX has anti-microbial activity against Doxycycline sensitive bacteria for approximately 30 days.

These findings support the claim of anti-bacterial activity of D-PLEX in prevention of infections in bone and soft tissue environment in human.

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

- According to local PK study in rabbit sternal model, the expected unbound Doxycycline concentrations at the administration site are maintained above the MIC value of clinically relevant sensitive bacterial species (e.g., *Staphylococcus aureus*: MIC of 1 µg/mL; Vibramycin, NDA 050007) for up to 30 days.
- D-PLEX ability to release Doxycycline for a period of at least 29 days in vivo was shown in rabbits after local administration of D-PLEX in the sternum and in rats after SC administration of D-PLEX.

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

- A safety and toxicity pivotal study in which D-PLEX was administered into a sternal defect in rabbits, shows that D-PLEX comparable on a mg/kg basis to twice the maximum D-PLEX applied dose to a 70-kg adult (i.e. 30 gr D-PLEX), demonstrates no systemic effects, no deleterious effects on healing of the sternal site, and no effect on the sternal bone's strength according to the 15-months available results.

[1] i.e. *Methicillin Resistant Staphylococcus Aureus (MRSA), Staphylococcus epidermitis coagulase negative (CoN), Enterococcus faecalis, Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae.*

- A safety study in which D-PLEX was administered onto the top of the sternum of rats (i.e. in between the muscles and the sternal bone) showed that D-PLEX comparable on mg/kg basis to [redacted] D-PLEX in a 70-kg adult, does not cause any systemic toxic effects nor local cytotoxic effects up to 3 months.
- 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 [redacted] D-PLEX in a 70-kg adult) to abdominal cavity is safe exposure.

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.

2.3 CLINICAL DATA

A Phase Ib/II clinical trial, conducted in Israel. The study 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 Standard of Care (SOC) vs. SOC alone, in the prevention of sternal wound infection post cardiac surgery for up to 24 weeks (6 months) following surgery. The recruitment to this study was completed with a total of [redacted] enrolled subjects: [redacted] subjects in part 1 & [redacted] subjects in part 2 of the study ([redacted] patients in the SOC arm and [redacted] patients in the treatment arm).

A total of [redacted] subjects were allocated to D- PLEX with SOC group, however, one subject withdrew consent before study treatment.

In a statistical report for the study the following were shown:

- A single patient in the SOC arm [redacted]) had superficial sternal wound infection (SSWI), on day 6 post-surgery. No surgical re-intervention was required due to sternal infection.
- No subjects in the treatment group experienced any sternal wound infection ([redacted]%).
- No hospitalizations, re admissions or surgical reintervention due to Sternal Surgical Site infection were reported in the study.
- No safety issues were indicated and no AE/SAE related to D- PLEX has been reported.
- [redacted] of the [redacted] subjects in the SOC group ([redacted]) and [redacted] of the [redacted] subjects ([redacted]) in the D-PLEX with SOC group experienced adverse events
- One subject in the D-PLEX plus SOC group died due to cardiogenic shock on day 6 post surgery, not related to the investigational product or study procedure.

Intense PK sampling was used to evaluate the plasma pharmacokinetic characteristics of the drug in a sub set of subjects in that study.

- Release of antibiotics from D-PLEX start upon administration
- Cmax of Doxycycline released from D-PLEX in sternal treated patients is at least 18 times lower than a systemic treatment of a comparable known oral drug
- Detectable plasma levels for three to four weeks from patients treated with the D-PLEX formulation.

- Based on the AUC (inf) parameters vs. the comparable known oral drug, most of the D-PLEX entrapped Doxycycline was released

2.3.1 Safety profile of Doxycycline

The antibiotic drug Doxycycline has been on the market for over 5 decades and its side effects are well established.

Information on adverse reactions of Doxycycline capsules USP are provided in the IB.

Furthermore, given the total dose of Doxycycline present in D-PLEX (i.e. maximum [redacted]), which is lower than the overall daily dose of systemically administered Doxycycline [redacted]), and given it is being gradually released over a period of approximately 3-4 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 Ib/II clinical study which was recently completed. It shows detectable plasma levels of Doxycycline for well into three to four weeks following administration of the D-PLEX formulation.

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

- Prevention of surgical site infection, which may occur within 30 days post-surgery.
- Significant improvement in prevention of surgical site infection when used in surgical procedures that hold high risk of infection such as: laparotomy and laparoscopy.
- Induces faster recovery and early return to normal activity as compared to SOC due to lower surgical site infection 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 result in local concentration above the minimal inhibitory concentration 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.
- Low potential risk for development of bacterial resistance.
- D-PLEX excipients are widely used in the medicinal or medical device industry, have an established history of safety and acceptable clinical use in contact with bone/ soft tissue and/or blood circulation and are biodegradable.

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

a) Potential contamination of microorganisms and pyrogens by the aseptic product.

Mitigation:

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

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

Mitigations:

- At subject level:
  In case of serious local toxicity induced by D-PLEX, removal and washing out the product will be done by the surgeon.
- At population level:
  - Close follow up of patients
  - Safety Monitoring

c) 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 incision and washing out the D-PLEX.

d) Potential of bacteria resistant to Doxycycline-treatment failure.
Mitigation:

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

e) 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 9.1.3.

f) Inadequate anti-bacterial activity of the product

Mitigations:

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

g) Re-use of D-PLEX between different patients, can lead to cross contamination.

Mitigations:

- D-PLEX vial will be re-allocated to a different patient 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.

h) Potential for permanent tooth discoloration or enamel hypoplasia - Tetracycline form stable complex in any bone formation tissue; Reversible decrease in fibula growth rate has occurred in premature infants receiving oral tetracyclines.

Mitigations:

- Exclusion of patients less than 18 years old from the clinical study.
- Exclusion of pregnant or lactating woman from the clinical study.

i) Photosensitivity reaction: exaggerated sun burn reaction.

Mitigations:

- Close follow up of patients

j) Transient hypercalcemia

Mitigation:

- Close follow up of patients.
- Blood levels of calcium are tested at screening and every visit after the procedure during the clinical study.

k) D-PLEX removed from the target organ following re-operation

Mitigation:

- D-PLEX should be re-applied during re-operation, if occurs within 72 hours of initial surgery, pending incision debridement. The amount to be re-used is pending size of the current incision.

2.6 SAFETY & EFFICACY SUMMARY

Based on the current data available from the one study with D-PLEX, in which [redacted] cardiac patients 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 patients undergoing abdominal surgery procedures, may be beneficial.

3 OBJECTIVES

- To assess the anti-infective efficacy of D-PLEX (in addition to SOC) over a period of 30 days post operation, by preventing surgical site infection (SSI) defined as superficial and deep abdominal wall surgical incision infection, compared to the SOC treated control arm.
- To assess the safety of D-PLEX.

4 STUDY ENDPOINTS

4.1 PRIMARY EFFICACY ENDPOINT

The following will be evaluated in this trial:
Infection rate as measured by the proportion of subjects with an SSI event, as determined by a blinded and independent adjudication committee, within 30 days post abdominal surgery.

Mortality from any reason within 30 days post index surgery, will be analyzed as treatment failure.

SSI is composed of Deep Incisional Surgical Site Infection (DSSI) and Superficial Incisional Surgical Site Infection (SSSI).

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

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

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

Anastomosis leak will not be accounted as an endpoint event.

4.2 SECONDARY EFFICACY ENDPOINTS:

Key endpoints:

- Number of hospitalization days post colorectal surgery due to SSI
- Average ASEPSIS assessment score during 30 days post-surgery.
- Number of Surgical Intervention due to SSI's

Additional endpoints:

- Incidence of SSSI rates during 30 days post-surgery.
- Incidence of DSSI rates during 30 days post-surgery
- Mortality rate within 60 days post abdominal surgery
- Determination of susceptibility to Doxycycline of any organisms recovered from an abdominal surgery incisional Infection Site.
- Number of overall hospitalization days within 60 days post-surgery (including days of readmission due to surgical site infection). Primary hospitalization for surgery and, if occurred, readmission due to abdominal incisional surgical site infection. Hospitalization days will be counted as aggregated overall hospitalization days per subject.
- Number of re-admissions due to surgical site infection.

- Number of antibiotic treatment days (all routes of administration) due to abdominal incisional surgical site infection (data will be aggregated and presented both for overall days of antibiotic treatment all routes of administration and IV administration treatment days).
- Time to surgical site infection post-surgery.

Identification of a Surgical site infection will be based on CDC/NHSN Patient Safety Component Manual criteria (January 2018, chapter 9).

4.3 END-POINTS ADJUDICATION COMMITTEE (EPAC)

A blinded and independent End-Point Adjudication Committee will be established to review and adjudicate study's primary efficacy end-point.

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

All information collected regarding efficacy (suspected SSI's, as per blinded investigator's assessments, as well as occurrences of deaths) will be reviewed by the End-Point Adjudication Committee. 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.

In case of dispute between investigator and the committee's assessment, End-Point Adjudication Committee's adjudication will prevail.

Summaries of the committee meetings will be provided periodically to the sponsor.

5 TRIAL DESIGN

5.1 OVERALL DESIGN

This is a phase II, prospective, multicentre, randomized, controlled, two arm, single blind study. The study population includes male and female, 18 years old and above at screening, undergoing elective abdominal colon surgery which involves resection and including at least 1 incision that is $\geq$ 5cm.

Subjects who meet the inclusion criteria and none of the exclusion criteria and provide a signed informed consent 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 blinded to the study arm.

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.

For subjects randomized to the control arm, the surgical treatment will be as per SOC as outline in this protocol.

Pre-operative care will be given to the study participants within 30-60 minutes prior to surgery and will include an antibiotic treatment from the 1st or 2nd generation of Cephalosporine family, plus Metronidazole.

Bowel preparation (mechanical only) and post-operative care for both arms will be performed per PI discretion and site SOC. Post-operative resumption of activity is at the discretion of investigator based on the subject's medical condition.

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 the controlled release antibiotic (Doxycycline) in the prevention of SSIs over a period of 30 days for the primary and secondary outcomes. All patients will be followed for an overall of 60 days for safety.

5.2 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.

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.

6 SELECTION OF SUBJECTS

About [redacted] eligible subjects will be enrolled and randomized 1:1 (D-PLEX therapy concomitant with SOC vs. SOC alone)

6.1 INCLUSION CRITERIA

Deviations from any inclusion criteria are not allowed because it 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 enrollment in the study must meet **all** of the following criteria:

1. Subjects undergoing elective abdominal colon surgery involving resection and ileocolonic, ileorectal, colocolonic or colorectal anastomosis or with a stoma, who are preoperative stable hemodynamically. In a laparoscopic surgery, an abdominal wall incision of at least ≥ 5 cm should be involved.
2. Male or non-pregnant female.
3. Female of childbearing potential should have a negative serum pregnancy test prior to index procedure.

Note: *All female of childbearing potential must agree to use a highly effective method of contraception (such as double barrier, oral or parenteral hormonal, intrauterine device and spermicide) consistently and correctly for the duration of the study.*

4. Subjects age 18years old and above at screening.
5. Subjects who sign a written informed consent.
6. Subjects who are willing and able to participate and meet all study requirements.
7. Survival expectancy of at least 60 days post randomization.

6.2 EXCLUSION CRITERIA

Deviations from any exclusion criteria are not allowed because it 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 scheduled for abdominal surgery which is classified as emergency.
2. Subjects with any preoperative infection that is currently being treated with antibiotics.
3. Subjects receiving any antibiotic therapy in the past 4 weeks prior to enrolment besides prophylaxis or antibiotic for the treatment of the disease that consists the indication for surgery.
4. Patients undergoing concomitant additional procedures besides colon resection surgery, e.g. Hyper-thermic Intraperitoneal Chemotherapy, liver resection etc. Female sterilization surgery (i.e. salpingo-oophorectomy, hysterectomy etc.), involvement of a small bowel procedure or Cholecystectomy may be allowed, pending an advanced consultation and approval from the sponsor.
5. Subject received chemotherapy within the last 4 weeks of surgery, or radiation for colorectal cancer to the abdomen area, prior to the planned abdominal surgery (neo-adjuvant treatment).
6. Subjects that received oral or IV Doxycycline during the past 4 weeks prior to screening.
7. Subjects with known sensitivity to Doxycycline and/or to the tetracycline family of drugs or to the D-PLEX's excipients.
8. Subjects with known allergies to more than 3 substances. (an allergy questionnaire will be filled in during the screening process).
9. Subjects with history of allergic/hypersensitivity reaction to any substance having required hospitalization and/or treatment with intravenous steroids/intramuscular epinephrine or in the opinion of the investigator the patient is at high risk of developing severe allergic/hypersensitivity reactions.
10. Subjects with uncontrolled Asthma (GINA III-IV).
11. Subjects with End Stage Renal Disease (ESRD/CKD stage 5).
12. Subjects with chronic urticaria.
13. Subjects diagnosed with TIA/CVA/ACS within the past 1 year prior to randomization.
14. Subjects that undergone any abdominal surgery and current planned surgery involves re-opening the scar of prior abdominal surgery.

15. Any subject with active malignancy or with malignancy that has not been in complete remission for at least 5 years. Excluding:
    * Subjects with potentially resectable non-metastatic colorectal cancer which consists the indication for surgery.
    * Subjects who have had carcinoma in situ of the cervix, squamous cell carcinoma of the skin and basal cell carcinoma of the skin
    * Subjects with a Non-violent cancer that does not require treatment 4 weeks prior to, and throughout the entire study duration.
16. Subjects with other concurrent severe and/or uncontrolled medical condition that could compromise participation in the study (e.g. non-GI active infection, uncontrolled diabetes, uncontrolled hypertension, congestive heart failure, unstable angina, ventricular arrhythmias, active ischemic heart disease, uncompensated cirrhosis, active upper gastrointestinal (GI) tract ulceration).
17. Psychiatric, addictive, or any other disorder that compromises ability to provide informed consent for participation in this study.
18. Chronic alcoholic or drug abuse subjects.
19. Pregnant or breast-feeding women or women of childbearing age who refuse or prohibited of using an effective contraceptive method of birth control (such as double barrier, oral or parenteral hormonal, intrauterine device and spermicide) throughout study participation including safety follow-up period.
20. Subjects that received any investigational drug within 30 days or 5½ half-lives of enrollment to the study (whichever is longer).
21. Subjects participating in any other interventional studies.
22. In the opinion of investigator, subject is not eligible to participate in the study and/or to comply with protocol requirements (e.g. due to a cognitive or medical condition).

7 RECRUITMENT

Patient recruitment will be conducted in general surgery departments at sites in Israel. Recruitment period is expected to take approximately 6 months.

8 STUDY PROCEDURES & SCHEDULE OF ASSESSMENTS

8.1 STUDY PROCEDURES

8.1.1 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 persons taking part in the study.

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

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

An original signed copy will be retained at the study site and 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 informed consent 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 that information shall be provided to the respective affected subject in written form. If relevant, all affected subjects shall be asked to confirm their continuing informed consent in writing.

8.1.2 Screen failure

Subjects who sign the informed consent but do not meet the eligibility criteria and were not randomized will be considered screen failures.

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

8.1.3 Randomization / enrollment

Upon confirmation that the subject fulfils all inclusion criteria and none of the exclusion criterion is met, subjects 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 surgery.

The following information for maintaining balance between the 2 arms must be entered into the system to obtain the treatment assignment:

- Gender
- Age (18-40, 41-65, 66and above)
- Open laparotomy surgery or laparoscopic surgery
- Surgery includes Colostomy - yes/no

Randomization will be done centrally using a randomization schedule generated by the study statistician, which will assign subjects in a 1:1 ratio to:

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

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

8.1.4 Blinding

This is a single blind clinical trial. Subjects will be blinded to the treatment arm assignment. The study site personnel present at the baseline procedure as well as the physician performing the procedure will be trained not to disclose the treatment arm to the subject, his/her family, to other health care providers not present during the surgery or to the study sponsor representatives.

Wound assessment throughout the study follow-up visits will be done by a blinded investigator.

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.

8.1.4.1 Emergency un-blinding

As a general rule, code breaks should occur only in exceptional circumstances when knowledge of the actual treatment is absolutely essential for further management of the patient. This is a single blinded study and as such, the investigator performing the procedure and study coordinator are expected to know the treatment arm of the subject and have access to this information through the eCRF, while all other medical staff should be kept blinded. If un-blinding is deemed necessary, the investigator can access information regarding the treatment arm information in the eCRF or the patient file.

In the rare case in which the investigator and study coordinator are unattainable or unable to access the eCRF, unblinding could be done by the study site personnel through a toll-free help line or through the local emergency number as the back-up system.

The Investigator is requested to maintain the blind as much as possible. Investigators are encouraged to discuss with a sponsor's representative if he/she believes that unblinding is necessary. The actual allocation must NOT be disclosed to the patient and/or other study personnel including other site personnel, monitors, corporate sponsors or project office staff.

The Investigator must report all code breaks (with reason) as they occur to the study CRA and on the corresponding CRF [case report form] page.

8.1.5 End point - assessment of infection rate

Primary Efficacy Endpoint:
Infection rate as measured by the proportion of subjects with an SSI event, as determined by the blinded and independent adjudication committee, within 30 days post abdominal surgery.
Mortality from any reason within 30 days post index surgery, will be analyzed as treatment failure.

Surgical site infection will be identified by the predefined criteria based on CDC/NHSN Patient Safety Component Manual (January 2018, chapter 9).
SSI is composed of Deep Incisional Surgical Site Infection (DSSI) and Superficial Incisional Surgical Site Infection (SSSI).

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

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

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

Anastomosis leak is excluded and will not be accounted as an endpoint event.

8.1.5.1 SSI criteria (based on CDC/NHSN January 2018, chapter 9,):

Superficial incisional SSI definition

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 followings:

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 based microbiologic testing method

c) Superficial incision that is deliberately opened

AND

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

d) Diagnosis of a superficial incisional SSI by the surgeon or attending physician or another designee.

Deep incisional SSI Definition

Must meet the following criteria:

The date of event for infection occurs within 30 days after he 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 by a microbiologic testing method

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

8.1.6 Management of suspected SSI

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

Action	Time Line
Collect Bacteriological Sample – **BEFORE** antibiotic treatment initiation and arrange for shipment into AML central lab for testing	Once suspicion arises
Collect Hematology/Chemistry Blood Samples	Immediately
Perform a clinical assessment of surgical incision area.	Immediately
Report infection suspicion through eCRF page	within 24h of knowledge
Send a detailed email to medical monitor/study group email.	Within 5 working days

8.1.7 Safety evaluation

The following safety parameters will be evaluated in this trial:

- Adverse events, physical examinations & vital signs.
- Incisional wound healing will be assessed by visual examination by investigators. A 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, urinalysis

8.1.8 Adverse events

The investigator and site staff are responsible for detecting, documenting, and reporting events that meet the definition of an AE, SAE or Event of Special Interest (i.e. SSI). Adverse events (AEs) will be collected once the abdominal wall incision closure process started, during the follow-up visits and until study termination at visit 7 (60 days after surgery).

Details on AE definition, assessment and reporting can be found in Section 10 "RECORDING & REPORTING OF ADVERSE EVENTS & REACTIONS".

8.1.9 Laboratory examinations

The following laboratory tests will be performed and then analyzed by the respective local laboratories:

a. Complete blood count (CBC) with differential white blood cell (WBC) count and platelet count; (all visits, except visit 2).
b. Urinalysis at screening and as per investigator discretion pH, Specific Gravity, Protein, Glucose, Bilirubin, Ketones, Nitrites, Leukocytes.
c. Blood chemistry: Glucose, Urea (BUN), AST (SGOT), ALT (SGPT), Total Bilirubin, Alkaline Phosphatase, Calcium, Potassium, Phosphorus, Sodium, Chloride, Total Proteins, Albumin, Serum Creatinine, Creatinine phosphokinase (CPK), C-reactive protein (CRP) & LDH.
   Coagulation (at screening only): INR, PT & (a)PTT.
d. Pregnancy test by serum at screening only, performed in females of child-bearing potential only. All females of child bearing potential must agree to use a highly effective method of contraception consistently and correctly for the duration of the study.
e. Rectal swab, at screening and at day 30. Kits will be provided. Samples will be forwarded to AML central lab for testing and results reported back to the referring investigator.

Any abnormal finding with respect to baseline, assessed by the investigator as clinically significant, should be recorded as adverse events in the relevant CRF form.

8.1.10 Bacteriological testing - (Should be taken in any case of suspected infection <u>before</u> starting Antibiotic treatment)

Bacteriological tests (from surgical site and/or surrounding soft tissue and/or 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 bacteriological specimen source should be indicated on source documents and CRF.

The test kit will be forwarded to AML central lab 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 AE in the relevant CRF section.

8.1.11 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 with respect to baseline, assessed by the investigator as clinically significant, should be recorded as an adverse event in the relevant CRF section.

8.1.12 Vital signs

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

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

All measurements and the time performed will be recorded in the eCRF.

Any abnormal finding, assessed by the investigator as clinically significant, should be recorded as an AE in the relevant eCRF section.

8.1.13 Electrocardiogram (ECG)

A standard 12-lead electrocardiogram (ECG) will be performed at screening, to identify subjects who have undiagnosed clinically significant anomalies that the investigator believes would preclude study participation.

The investigator may choose to use a copy of an ECG print-out/results done up to 90 days prior to the planned surgery.

The ECG review is under the investigator's responsibility.

Any abnormal finding, assessed by the investigator as clinically significant, should be recorded in the relevant eCRF form and source documents.

8.1.14 Medical history and prior concomitant medications

Demographic data and a complete medical history of past, 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.

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

Subjects will be permitted to receive concomitant therapy as medically required, except for systemic Doxycycline or steroids use that in the opinion of the investigator puts the patient is at high risk of developing severe allergic/hypersensitivity reactions.

A record of all concomitant prescription medications will be maintained.

8.1.15 Allergy questionnaire

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

8.1.16 Assessment of surgical site

A blinded investigator will inspect the surgical site and asses the healing of the surgical wound. Special attention is to be paid for signs of infection. The Investigator should follow the protocol guidelines for assessment of SSI (see above section 8.1.5).

Two questionnaires will be completed: SSI assessment questionnaire per CDC criteria, and an ASEPSIS scoring.

Every effort must be made to assess the incisional surgical site at every visit. In case a subject is un willing or un able to attend a scheduled visit, a phone conversation should be held with him, his 1st degree relative or community physician and assessment of the wound per CDC should be done.

8.1.16.1 ASEPSIS: (Additional treatment, Serous discharge, Erythema, Purulent exudate, Separation of deep tissue, Isolation of bacteria, Stay duration as inpatient) scoring method.

ASEPSIS is 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 anesthesia, isolation od pathogenic bacteria and stay as inpatient.

8.1.17 Pharmacokinetics

Doxycycline pharmacokinetic sampling will be collected from three subsets administered with different dose of D-PLEX (whole 1, 2 or 3 vials) in at least 24 patients (i.e., 8 patients per subset, pending subset recruitment).

Pharmacokinetic samples of 4ml blood will be collected at each PK time point, prepared and shipped to a central laboratory.

Visit / Day	Time after surgery	Comments
Surgery - Day 0	Pre-application of D-PLEX	Subject hospitalized
(Visit 2)	After D-PLEX Application; 2 hours 4 hours 6 hours 8 hours 12 hours	Subject hospitalized
Day 1 (Visit 3)	24 hours	Subject hospitalized
Day 2	48 hours	Only if subject still hospitalized
Day 3	72 hours	Only if subject still hospitalized
Day 5 (Visit 4)	Day 5	
Day 14 (Visit 5)	Day 14	
Month 1 (Visit 6)	Month 1	

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

For patients where D-PLEX was re-applied due to re-intervention, PK sampling should be stopped and the reason stated on their PK collection forms.

Details for the preparation and shipment of samples are attached as appendix number 1.

8.2 STUDY VISITS

8.2.1 Visit 1: Screening period (day -21 - 0)

Patient will be screened for study eligibility at the clinic within 21 days prior to surgery (Day -21 - 0). The following screening assessments will be performed:

- Sign an Informed Consent form before any of the study related procedures are performed
- Assign (from the eCRF) a sequentially ordered patient number for the site. If a patient is withdrawn from the study or fails to undergo the treatment procedure, the patient number cannot be reassigned

- Record demographics (including age, gender, etc.)
- Record medical history
- Complete allergy questionnaire
- Record concomitant medications
- Measure vital signs (Height, weight, Temp., heart rate & blood pressure)
- Perform physical examination
- Perform blood laboratory tests *
- Perform 12-lead ECG**
- Perform Urine analysis *
- Perform a rectal swab for bacteriology
- Record baseline safety (any future planned elective procedure should be recorded so it will not be counted later on as an AE or SAE as applicable)
- Eligibility determination according to inclusion and exclusion criteria

* Blood tests and Urinalysis done at the medical center's lab within 21 days prior to surgery may be used for eligibility determination.

** ECG done within the last 90 days prior to surgery can be used for eligibility determination.

8.2.2 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 prophylactic antibiotic treatment used in this study is consistence and standardized for all sites in the clinical study and is composed of antibiotic treatment from the 1st or 2nd generation of Cephalosporine family, plus Metronidazole.

Bowel preparation (mechanical only) and post-surgery treatment will be done as per each site's SOPs.

The following will be performed during visit 2:

- Randomization***
- Vital signs
- Pre-application Doxycycline PK sampling (for applicable subjects)
- Abdominal Surgery
- D-PLEX administration at the stage of closure of the abdominal wall surgical incision
- Post-application Doxycycline PK sampling (at 2h, 4h, 6h, 8h & 12h) – selected subjects
- Adverse Events assessment and recording
- Concomitant Medication Review

*** for surgery planned for early morning time, randomization via the eCRF could be done a day in advance.

8.2.3 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 are at the discretion of investigator based on subject medical condition.

The subsequent follow up visits (visits 3-5) will take place at 1 day, 5 days and 14 days 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 wound healing – Including visual examination and wound assessment questionnaires.
- Laboratory examinations
- Post-application Doxycycline PK sampling – selected subjects
- Adverse Events assessment and recording
- Concomitant Medication Review
- Physical examination [at visit 6 (D30)]
- Rectal swab for comparable bacteriology [at visit 6 (D30)]

Urinalysis will be performed only at the discretion of the investigator.

Bacteriology cultures will be taken if there is wound discharge.

8.2.4 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. Vital signs, adverse events and concomitant medications will be assessed. 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.

8.2.5 Visit 7 - end of study (60 days post-surgery)

- Vital signs
- Assessment of surgical site
- Laboratory examinations
- Adverse Events assessment and recording.
- Concomitant Medication Review
- Physical examination

Table 1 details all scheduled/planned assessment at these visits.

8.3 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[8])	Visit 5 Day 14 (± 3 days)	Visit 6 Day 30 (± 3 days)	Visit 7 Day 60 - Termination (± 7 days)
Informed Consent	X						
Medical History & Allergy questionnaire completion	X						
General Eligibility Criteria	X	X[1]					
Physical Exam	X					X	X
12-Lead ECG[2]	X						
Vital Signs (blood pressure, HR, body temperature)	X	X	X	X	X	X	X
Weight & Height	X						
Pregnancy Test (serum)	X						
Assessment of Surgical Site			X	X	X[6]	X[6]	X[6]
Blood Tests (hematology, chemistry[3])	X		X	X	X	X	X
Rectal swab[7]	X					X	
Doxycycline PK Sampling[4]		X	X	X	X	X	
Urinalysis [2,5]	X						
Adverse Events		X	X	X	X[6]	X[6]	X[6]
Concomitant Medications	X	X	X	X	X	X	X
D-PLEX Administration		X					

Notes:

1. Confirmation of Eligibility
2. Could be performed within 90 days prior or at visit 2 (before surgery).
3. Blood chemistry: Glucose, Urea (BUN), AST (SGOT), ALT (SGPT), Total Bilirubin, Alkaline Phosphatase, Calcium, Potassium, Phosphorus, Sodium, Chloride, Total Proteins, Albumin, Serum creatinine, Creatinine phosphokinase (CPK), C-reactive protein (CRP). Coagulation tests (PT, INR, PTT) only at Screening.
4. PK timelines include a pre-surgery sample and 2, 4, 6, 8, 12, 24, 48, 72h, D5, D14 & D30.
5. Urinalysis will be done at screening visit and at the discretion at the investigator during the other study visits.
6. In case the subject is unable to attend a visit, information related to his wound assessment (as per PolyPid's designated form) should be obtained in a phone conversation with him/his community physician/a 1st degree relative.
7. To be forward to and analyses by a central lab
8. If D5 occurs on a Saturday/holiday.

8.4 DEFINITION OF END OF TRIAL

Total duration of the study is expected to be approximately 8 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 patient.

8.5 DISCONTINUATION/WITHDRAWAL OF PARTICIPANTS & 'STOPPING RULES'

While study withdrawal is not encouraged, subjects may choose to withdraw from the study at any time, with or without reason and without prejudice to further treatment. Moreover, subject's participation in any clinical trial is voluntary and the subject has the right to withdraw at any time without penalty or loss of benefit.

In the event that a subject withdraws from the study, 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 withdrawal (if given) will be recorded on the appropriate eCRF and subject's medical records in all cases of withdrawal. 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 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 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.

9 NAME & DESCRIPTION OF ALL DRUGS USED IN THE TRIAL

D-PLEX is the investigational medicinal product 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.

9.1 STUDY TREATMENT D-PLEX

9.1.1 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 base. Doxycycline is being constantly released for approximately 3-4 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 [redacted] vial contains [redacted] g of D-PLEX.

9.1.2 Product Composition

D-PLEX contains the following substances:

Substance	Description
Doxycycline Hyclate	Antibiotic
Beta-Tri Calcium Phosphate	Release system
Polymer and Lipids	

9.1.3 Product Handling (storage & preparation)

D-PLEX is supplied as sterile powder to be reconstituted to paste in the operating room, using standard aseptic techniques and is intended for a single administration.

D-PLEX cannot be injected!

Each [redacted] vial contains [redacted] 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:

Step 1:

NOTE: This step should be performed separately for each vial until its content is poured into a sterile bowl (sections 1-10). The content of all the vials intended to be administered should be poured into the same bowl. It should be done by a sterile team member.

- Remove D-PLEX product from the refrigerator.
- Open the cartonto expose the amber glass vial. **Caution: The outer surface of the vial <u>is not sterile</u>. Only the vial content is sterile.**
- Make sure that the integrity of the box and vial was not damaged. **If it was - do not use it.**
- Take out the vial from the opened box.
- Put a pair of **<u>sterile</u>** gloves.
- Wipe the entire vial (including the cap) with alcohol swabs.
- Vigoursly shake the vial until a homogeneous powder is received.

- Remove the vial cap by flipping the cap up and tearing it counter clockwise, according to the arrows stamped on the cap.
- Open the vial slowly close to a sterile mixing surgical bowl, using standard aseptic techniques.
- Pour **the entire** content of the vial ([redacted]) into the sterile mixing bowl using standard aseptic techniques.

  Make sure that only the vial neck is found above the sterile mixing bowl.
- Repeat steps 1-10 above for the second vial, if two vials are needed for administration, and also for the third vial, if a total of three vials are needed for administration. **The content of all the vials intended to be administered should be poured into the same bowl.**

Step 2:

NOTE: This preparation step should commence upon surgeon confirmation. It should be done by a sterile team member.

- Slowly hydrate the D-PLEX powder in the bowl with the following volume of sterile saline:
  D-PLEX powder from 1 vial should be hydrated with 2ml of sterile saline.
  D-PLEX powder from 2 vials should be hydrated with 3.5ml of sterile saline.
  D-PLEX powder from 3 vials should be hydrated with 5ml of sterile saline.
  Do not over hydrate.
- Mix D-PLEX powder, with the sterile saline using sterile spatula, for approximately 1 minute, until uniform paste appearance is reached.

Step 3:

Should be done by the operating surgeon.

- **Administer** the whole quantity of the paste directly within the surgical site: between the two halves of the surgical incision **immediately** after hydration and mixing, using the same spatula.
- Discard the remaining of D-PLEX per local environmental standards.
- Packages (vial and box) should be kept for inventory documentation purposes.

9.1.4 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 surgical incision length. A maximum of three vials [redacted] may be administered. Therefore, the total amount of Doxycycline free base for a single surgical procedure with D-PLEX can reach up to [redacted] mg.

The individualized dose will follow the following scheme:

Surgical incision length (cm)	Max number of vials to apply*
5-10	1
11-20	2
>21	3

* Used D-PLEX paste will be measured and documented in the eCRF.

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

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.

9.1.5 Contra-Indications

Please refer to the exclusion criteria (section 7.2).

9.1.6 Use in Specific Populations

Please refer to the inclusion criteria in D-PLEX (section 7.1).

9.1.7 Warnings and Precautions

9.1.7.1 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.

9.1.7.2 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 patient.
- Avoid extended exposure to extreme heat, or humidity.

9.1.8 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 patient (i.e. more than [redacted] per patient) 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 patient.

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 over dosage. 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.

9.1.9 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.

9.2 CONCOMITANT MEDICATION

9.2.1 Medication(s)/Treatment(s) Permitted

- Concomitant Medications

  All currently used concomitant medications, as well as all medication taken/prescribed to the patient throughout the study will be recorded in the case report form (eCRF).

- Antibiotics Treatment per SOC Allowed in this Protocol:

  Subjects will receive prophylactic IV antibiotics per protocol. Additional antibiotics (excluding Doxycycline) can be administered (per need), according to investigator discretion.

  Any additional concomitant medication for the treatment of concurrent illnesses are allowed at the discretion of the treating physicians.

9.2.2 Medication(s) prohibited during the trial

- Additional antibiotics (including antimicrobial sutures), except those used as part of the post-operative SOC or approved by the study investigator.

- High steroids dose that in the opinion of the investigator puts the patient at high risk of developing severe allergic/hypersensitivity reactions
- Doxycycline IV/PO administration is not allowed in this protocol during the entire trial period.

9.3 PREPARATION & LABELING OF INVESTIGATIONAL PRODUCT (IP)

The investigational product is manufactured aseptically at Nextar, at a Grade A (ISO 5) clean room. The investigational product will be provided to the investigational site by PolyPid Ltd. via IMP Ltd. depot, once Ethics Committee approval of the protocol and the Informed Consent Form, 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 depyrogenated inert glass amber vial ([redacted]), and each product vial contains [redacted] of D-PLEX. The sterile vial is packaged in a carton box.

Preparation and labelling of the investigational medicinal products will be completed in accordance with the relevant GMP guidelines.

Information on the Storage and Handling of the IP can be found in section 10.1.3 of this protocol.

9.4 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 PolyPid 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 instructions.

10 RECORDING & REPORTING OF ADVERSE EVENTS & REACTIONS

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

10.1 DEFINITIONS

10.1.1 Adverse event (AE)

Any untoward medical occurrence in a subject participating a clinical trial, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

NOTE: An AE can therefore be any unfavourable and unintended sign (including an abnormal 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/SAE).

"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 is 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.

10.1.2 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.

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

10.1.3 Serious adverse event (SAE)

A serious adverse event is any untoward medical occurrence that, at any dose:

- 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 were 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 AE's. If a complication prolongs hospitalization or fulfills 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 AE.

- 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.
- 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.

10.1.4 Unexpected adverse event

An adverse reaction which the nature and/or severity of which is not consistent with the information about the medicinal product in question set out in the investigator's brochure relating to the trial in question.

10.2 ASSESSMENT OF ADVERSE EVENT

Adverse event and serious adverse events will be reported in accordance

10.2.1 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.

10.2.2 Causality

Investigator'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 case report form. Investigator may change the causality assessment based on new information as additional tests, medical history etc. will become available.

The following categories will be used to define the causality of the adverse event:

Category	Definition
Related	There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out.
Possibly Related	There is some evidence to suggest a causal relationship (e.g. the event occurred within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant events).
Unlikely Related *	Causal relationship improbable, and other interventions or underlying disease provide plausible explanations.
Not related	There is no evidence of any causal relationship.

** Note: in this protocol "Unlikely Related" will be regarded as "Not Related" for the purpose of AE assessment and reporting.*

10.2.3 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 Sequalea	Subject has recovered, but some signs/symptoms persist. The sequelae should not be reported as separate event but should be described in the "General Narrative Comments" section of the AE/SAE reporting page.
Resolved	Subject has recovered and all AE/SAE's signs and symptoms resolved.
Death	Only one event should be recorded as the fatal event. The SAE which was the primary cause of death and/or was 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".

10.2.4 Expectedness

Category	Definition
Expected	An adverse event 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 adverse event 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.

10.2.5 Seriousness

Seriousness as defined for an SAE in section 10.1.3.

10.3 DOCUMENTATION AND REPORTING OF SAE

All serious adverse events will need to be reported to the sponsor within 24 hours of knowledge, according to local regulations.

SAEs will be reported through the study eCRF. In case the eCRF is not available, the Principal Investigator will complete the sponsor's serious adverse event form and the form will be emailed to the sponsor to the following email: safety@polypid.com within 24 hours of knowledge.

It is the Principal Investigator's responsibility to respond to any SAE queries raised by the sponsor as soon as possible.

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

1. SAE event description
2. SAE onset date
   Report the start date the event became SAE. If signs and symptoms started prior to event turning into SAE, record them as an AE.
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 (Not Related, Possibly Related, unlikely related or Related)
8. Action taken to resolve the SAE (Treatment, Other Action)
9. Outcome (Ongoing, Resolved with Sequelae, Resolved, Death, Lost to Follow Up)
10. Narrative of the event

10.4 TIME PERIOD AND FREQUENCY FOR DETECTING AEs, SAEs AND EVENTs OF SPECIAL INTEREST

The investigator and site staff are responsible for detecting, documenting, and reporting events that meet the definition of an AE, SAE or Event of Special Interest (i.e SSI).

Adverse events (AEs) will be collected from the time of randomization and during follow-up visit until study termination at visit 7 (60 days after surgery).

Serious adverse events (SAEs) will be collected over the same time period as stated above for AEs. 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.

All SAEs will be reported to sponsor within 24 hours, as indicated in the table below.

	Initial Reports		Follow-up Reports	
Type of Event	**Time Frame from Knowledge**	**Documents**	**Time Frame from Knowledge of new information**	**Documents**
All SAEs	24 hours	SAE data collection tool	24 hours	Updated SAE data collection tool
All AEs	5 days	AE data collection tool	5 days	AE data collection tool
Any AE/SAE related or possibly related to IP	24 hours	AE/SAE data collection tool	24 hours	AE/SAE data collection tool
All Suspected and/or Confirmed SSI events	24 hours	SSI data collecting tool	24 hours	SSI data collecting tool

If the investigator suspects that the subjects' disease has progressed faster due to the administration of the investigational medicinal product (IP), then he will record and report this as an adverse event.

Clinically significant abnormalities in the results of objective tests (e.g. infection status, laboratory variables, ECG, etc.) will also be recorded as adverse events.

All adverse events will be recorded with clinical symptoms and accompanied with a simple, brief description of the event, including dates as appropriate.

10.4.1 Procedures for recording & reporting serious adverse events

All serious adverse events will be recorded in the hospital's patient notes/system, the eCRF, and the sponsor's SAE log.

10.4.2 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.

10.5 REPORTING OF SUSARS

The sponsor will notify the Investigator of all SUSARs. SUSARs that are fatal or life-threatening must be notified to the Investigator within 7 days after the sponsor has learned of them. Other SUSARs must be reported to the Investigator within 15 days after the sponsor has learned of them.

Investigator will forward SUSARs that occurred at his centre to the EC/IRB in a timely manner. All other SUSARs will be forwarded to EC/IRB periodically (at least every 6 months).

10.6 DEVELOPMENT OF SAFETY UPDATE REPORTS

The sponsor will provide all ECs/IRBs and the Ministry of Health/Regulatory Authority with a Safety Report which will be written in conjunction with the trial team and the Sponsor's office and which will be updated yearly on the anniversary of receipt of hospital's approval to conduct the study (Form # 7).

10.7 THE TYPE & DURATION OF THE FOLLOW-UP OF SUBJECTS AFTER ADVERSE EVENTS

All AE/ SAEs not related to the study product should be followed up until resolution, permanent outcome or study termination, whichever is earliest.

All AE/SAEs suspected to be related to the study product should be followed up until resolution or permanent outcome.

SAE- If information is not available at the time when the first report becomes available, the investigator should fill out a follow-up SAE report at a later date and send to the Sponsor. (Per the instructions of EDC completion).

It is the responsibility of investigators to inform their Ethics Committee of SAEs (whether IP related or not) as required by their Ethics Committee procedure.

Any AE related to the IP (D-PLEX) will need to be reported to the Sponsor irrespective of how long after IP administration the event has occurred.

11 NOTIFICATION OF SERIOUS GCP BREACHES AND/OR PROTOCOL VIOLATION

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

A "serious breach" is a breach which is likely to effect to a significant degree –

(a) The safety or physical or mental integrity of the subjects of the trial; or

(b) The scientific value of the trial.

It is within the site's PI 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

CONFIDENTIALITY

The Electronic Case Report Form (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.

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 provide clinical monitoring throughout the study.

It will be the responsibility of the investigator to ensure the accuracy of all data entered in the eCRFs.

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 manor, 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. In no case can the eCRF be considered as source data for this trial.

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:

a) Subject files containing copies of completed case reports and supporting documentation and the signed Informed Consent forms.

b) 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.

c) 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

14.1 SAMPLE SIZE AND POWER

The study will enrol a total of [redacted] subjects, with [redacted] subjects allocated to each treatment group. This sample size was chosen by the Sponsor to collect adequate data for evaluating the primary study objectives. This study is not powered to test formal statistical hypotheses. However, exploratory sample size calculations show that a sample of [redacted] subjects provides [redacted] power to detect an [redacted] decrease in in the SSI rate ([redacted] versus [redacted]) at a two-sided α=0.10 level of significance.

A blinded sample size re-assessment to evaluate the blood will be conducted after a total of about N =[redacted] and [redacted] subjects, have been assessed for primary efficacy endpoint. The maximal sample size allowed to upsize the study will not exceed [redacted] subjects.

14.2 ANALYSIS POPULATIONS

The following analysis populations will be defined for the study:

Intention to treat (ITT) set - The ITT analysis set will consist of all subjects who have been randomized to receive either D-PLEX or SOC. In this analysis set, treatment will be assigned based on the treatment to which patients were randomized, regardless of which treatment they actually receive.

Safety analysis set - The safety analysis set will consist of all patients who have been randomized and treated. In this population, treatment will be assigned based upon the treatment patients actually receive, regardless of the treatment to which they were randomized.

Modified intention to treat (mITT) set - The mITT analysis set is a subset of the ITT set and will consist of all patients randomized and treated with D-PLEX or SOC, for whom there is at least one valid post-surgical observation on the primary endpoint and meet all inclusion/exclusion criteria.

The analysis of primary and secondary efficacy endpoints will be performed on the ITT and mITT populations. 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 (mean, median, sample size, standard deviation, minimum, and maximum). Categorical variables will be summarized via counts and percentages.

14.3.2 Primary Endpoint Analysis

The number and proportion of subjects with at least one SSI, as determined by a blinded and independent adjudication committee, within 30 days post abdominal surgery will be tabulated per treatment group. A 95% confidence interval using exact binomial methods will be constructed for each proportion.

Missing data for the primary efficacy endpoint will be imputed according to the methods described in Section 14.3.5.

14.3.3 Secondary Endpoint Analysis

Secondary endpoints analyses will be defined in the Statistical Analysis Plan (SAP) for the study. The SAP will be written and finalized prior to database lock.

14.3.4 Safety Evaluations

Prior to analysis, all AEs will be coded using the MedDRA coding dictionary. The number and percentage of subjects with at least one AE will be summarized by system organ class (SOC) and by preferred term (PT) within SOC for each treatment group. A similar summary will be provided related to subjects with at least one serious AE (SAE). In addition, tables will be constructed to summarize the Treatment Emergent Adverse Events (TEAEs) by relation to study treatment and by severity.

Summary statistics on the raw and change from baseline data will be computed for each vital sign parameter and continuous laboratory parameter by time point for each treatment group. Shift

tables will be constructed to show the changes in laboratory findings from pre-treatment to each post-treatment time point.

A physical exam (PE) shift table will be constructed for each treatment group to identify the changes in body system findings from pre-treatment to each post-treatment time point where PE is performed (visits 6 and 7).

Further analyses of safety variables for this study will be described in the SAP.

14.3.5 Handling of Missing Data

Missing data for the primary efficacy endpoint will be imputed for the ITT analysis set. Deaths occurring within the first 30 days post abdominal surgery will be counted as treatment failures in the calculation of the primary efficacy endpoint for each treatment group. Subjects who have missing primary efficacy endpoint data due to other reasons will also be counted as treatment failures in the calculation of the primary efficacy endpoint for each treatment group.

There will be no imputation of missing data for the mITT analysis of the primary endpoint, and there will be no imputation of missing data for all other study endpoints (i.e., a complete case analysis will be used).

14.3.6 Interim Analyses

No formal interim analyses are planned for this study.

A complete statistical report including safety and efficacy endpoints will be prepared when all the subjects complete their follow-up period and all safety and efficacy evaluations are completed.

15 DIRECT ACCESS TO SOURCE DATA DOCUMENTS

As required, the principal investigator 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, informed consent form 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 centres' 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 PolyPid 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 satisfy regulatory, ethical, and PolyPid 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

To ensure compliance with GCP and all applicable regulatory requirements, PolyPid may conduct a quality assurance assessment and/or audit of the site records. Regulatory agencies may conduct a regulatory inspection at any time during or after completion of the study.

In the event of an assessment, audit or inspection, the investigator (and institution) must 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 preventative 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 PolyPid and shall be held in strict confidence along with all information furnished by PolyPid, 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 PolyPid.

Written permission to the Investigator will be contingent on the review by PolyPid of the statistical analysis and manuscript and will provide for nondisclosure of PolyPid 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 favorable 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.

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Appendix 1: 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 polypropylene 2ml Eppendorf tubes. Try to keep equal amounts of plasma with the 2 tubes.
    - Mark these 2 tubes as: A (primary) and B (back-up), complete the appropriate label and place them onto the 2ml tubes.
6. Plasma samples must be stored at 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: XenoBiotic Laboratories, Inc.

Back-up Sample Shipment

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