

 POLYPID OPTIMIZED THERAPEUTICS	D-PLEX -302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 1 of 98

Study Number:	Study D-PLEX 302
Full Title of Trial	A Phase III, Prospective, Multinational, Multicenter, Randomized, Parallel Controlled, Two arms, Single Blind, Study to Assess the Efficacy and Safety of D-PLEX Administered Concomitantly with the Standard of Care IV Prophylactic Antibiotic Treatment (SOC) vs. SOC only, in Prevention of Post-Cardiac Surgery Sternal Infections.
Short Title	Efficacy and Safety of D-PLEX in the Prevention of Sternal Infection Post Cardiac Surgery.
Version of Protocol	Version 06
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Sponsor Protocol Number	D-PLEX 302
Phase of Trial	Phase III
Site(s)	About [REDACTED] centers in the US, Europe and Israel
Study Principal Investigator	[REDACTED] Mayo Clinic 200 First Street SW Rochester, MN. 55902
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	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 2 of 98

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Protocol Version: 06

Protocol Date: 23 September 2020

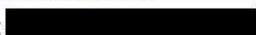
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	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 3 of 98

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	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 4 of 98

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	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 5 of 98

INVESTIGATOR SIGNATURE

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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 IMP and the conduct of the trial.

I will conduct the trial in accordance with the clinical trial protocol, Good Clinical Practice 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.

Principle Investigator Name (print)

Principle Investigator (signature)

Date

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	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 6 of 98

DOCUMENT HISTORY

Revision	Date reviewed/revised	Changes
Version 01	NA	Initial Release
Version 02	01 January 2019	Updated in accordance with FDA comments
Version 03	27 January 2019	Correction of typographical errors
Version 04	03 March 2019	Updates
Version 05	26 March 2020	Updated in accordance with CTA (VHP) comments and additional administrative updates
Version 06	23 September 2020	Update PK plan

DOCUMENT CHANGE HISTORY

Revision	Changes	Reason for changes
Version 06	Summary, Section 5.2, 8.10.1 – PK plan was updated	Population PK will not be performed as Thorough PK Analysis for [REDACTED] subjects is performed and is sufficient to complete PK profile of D-PLEX. D-PLEX from PolyPid manufacturing site only will be used in this study.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 7 of 98

TABLE OF CONTENTS

TABLE OF CONTENTS	7
LIST OF ABBREVIATIONS.....	11
1 TRIAL PERSONNEL.....	13
2 SUMMARY	14
3 INTRODUCTION	27
3.1 BACKGROUND	27
3.2 NON-CLINICAL STUDIES.....	30
3.3 CLINICAL DATA	32
3.4 POTENTIAL BENEFITS OF D-PLEX FOR PREVENTION OF POST CARDIAC SURGERY STERNAL INFECTIONS.....	33
3.5 SAFETY & EFFICACY SUMMARY	35
4 OBJECTIVES.....	35
5 EFFICACY & SAFETY ENDPOINTS.....	35
5.1 EFFICACY ENDPOINTS.....	35
5.2 SAFETY ENDPOINTS	36
5.3 TRIAL DESIGN.....	37
5.4 DESCRIPTION OF THE DURATION OF TREATMENT, SUBJECT PARTICIPATION AND TRIAL FOLLOW-UP	38
6 SELECTION OF SUBJECTS	38
6.1 INCLUSION CRITERIA	38
6.2 EXCLUSION CRITERIA.....	39
6.3 CONTRACEPTIVE METHODS	41
7 RECRUITMENT	42
8 STUDY PROCEDURES	42
8.1 INFORMED CONSENT PROCEDURE	42
8.2 SCREEN FAILURE.....	42
8.3 RANDOMIZATION	42
8.4 SUBJECT ENROLLMENT.....	43
8.5 BLINDING	43

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 8 of 98

8.6	END POINT - ASSESSMENT of STERNUM INFECTION	44
8.7	SAFETY EVALUATION	47
8.8	LABORATORY EXAMINATIONS	48
8.9	ASEPSIS	50
8.10	PHARMACOKINETICS.....	50
8.11	MEDICAL HISTORY AND PRIOR CONCOMITANT MEDICATIONS.....	52
8.12	VITAL SIGNS (ALL VISITS)	52
8.13	LABORATORY EXAMINATIONS	52
8.14	PHYSICAL EXAMINATION (SCREENING & VISITS 6, 8 & 10)	52
8.15	ELECTROCARDIOGRAM (ECG) - (ONLY AT SCREENING)	53
8.16	ALLERGY QUESTIONNAIRE (ONLY AT SCREENING).....	53
8.17	SURGERY – INDEX PROCEDURE - VISIT 2	54
8.18	SUBSEQUENT VISITS AND ASSESSMENTS (VISITS 3 – 10).....	54
9	SCHEDULE OF ASSESSMENTS	55
9.1	VISIT 1 (SCREENING, DAY -7)	55
9.2	VISIT 2 (SURGERY, DAY 0)	56
9.3	FOLLOW UP VISITS 3-9	56
9.4	VISIT 10 (END OF STUDY, 6 MONTHS)	56
9.5	TABLE OF STUDY ASSESSMENTS.....	57
9.6	DISCONTINUATION/WITHDRAWAL OF PARTICIPANTS & ‘STOPPING RULES’	60
10	NAME & DESCRIPTION OF ALL DRUGS USED IN THE TRIAL.....	60
10.1	STUDY TREATMENT D-PLEX.....	61
10.2	WARNING AND PRECAUTIONS	62
10.3	PRODUCT HANDLING (STORAGE & PREPARATION)	62
10.4	CONCOMITANT MEDICATION	65
10.5	PREPARATION & LABELING OF INVESTIGATIONAL MEDICINAL PRODUCT (IMP)	66
10.6	DRUG ACCOUNTABILITY	66
11	RECORDING & REPORTING OF ADVERSE EVENTS & REACTIONS	67
11.1	DEFINITIONS	67

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 9 of 98

11.2	ASSESSMENT OF ADVERSE EVENT	69
11.2.1	SEVERITY	69
11.2.2	CAUSALITY	69
11.2.3	OUTCOME	70
11.2.4	EXPECTEDNESS	71
11.2.5	SERIOUSNESS	71
11.3	DOCUMENTATION AND REPORTING OF SAE	71
11.4	TIME PERIOD AND FREQUENCY FOR DETECTING AEs, SAEs AND EVENTs OF SPECIAL INTEREST (STERNAL INFECTION).....	72
11.5	REPORTING OF SUSARs	74
11.6	DEVELOPMENT OF SAFETY UPDATE REPORTS	74
11.7	THE TYPE & DURATION OF THE FOLLOW-UP OF SUBJECTS AFTER ADVERSE EVENTS.....	74
12	NOTIFICATION OF SERIOUS BREACHES TO GCP AND/OR PROTOCOL VIOLATION.....	75
13	DATA MANAGEMENT & QUALITY ASSURANCE.....	75
13.1	CONFIDENTIALITY	75
13.2	DATA COLLECTION TOOLS & SOURCE DOCUMENT IDENTIFICATION	75
13.3	DATA HANDLING & ANALYSIS	75
14	RECORD KEEPING & ARCHIVING	76
15	STATISTICAL CONSIDERATIONS	77
15.1	STUDY DESIGN AND RANDOMIZATION PROCEDURE	77
15.2	SAMPLE SIZE CONSIDERATION	77
15.3	INTERIM ANALYSIS	79
15.4	ANALYSIS SETS	80
15.5	PATIENT DISPOSITION	80
15.6	DEMOGRAPHIC AND BASELINE CHARACTERISTICS	80
15.7	END POINTS	81
15.8	CONTROLLING OF TYPE I ERROR	82
15.9	STATISTICAL ANALYSES.....	82
16	NAME OF COMMITTEES INVOLVED IN TRIAL	86
16.1	DATA MONITORING COMMITTEE (DMC).....	86

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 10 of 98

16.2	END-POINTS ADJUDICATION COMMITTEE (EPAC)	86
17	DIRECT ACCESS TO SOURCE DATA DOCUMENTS	87
18	ETHICS & REGULATORY REQUIREMENTS	87
19	QUALITY CONTROL (STUDY MONITORING).....	87
20	QUALITY ASSURANCE	88
21	INSURANCE	88
22	PUBLICATION POLICY.....	88
23	STATEMENT OF COMPLIANCE	89
24	REFERENCES	90
25	APPENDICES.....	93
25.1	ASEPSIS SCORE ASSESSMENT	93
25.2	PAIN VAS SCORE	93
25.3	MODIFIED VANCOUVER SCAR SCALE	94
25.4	DOCUMENT CHANGE HISTORY	95

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 11 of 98

LIST OF ABBREVIATIONS

ACS - Acute Coronary Syndrome

AE - Adverse Event

AR - Adverse Reaction

ASC/AST - Active Surveillance Culture/Testing

BMI - Body Mass Index

CABG - Coronary Artery Bypass Grafting

CDC - Center for Disease Control

COPD - Chronic Obstructive Pulmonary Disease

eCRF - Electronic Case Report Form

CVA - Cerebro Vascular Accident

DSWI - Deep Sternal Wound Infections

EC - Ethics Committee

ECG - Electrocardiograph

g - gram

GCP - Good Clinical Practice

GMP - Good Manufacturing Practice

IB - Investigator's Brochure

ICF - Informed Consent Form

ICU/CCU - Intensive Care Unit/Critical Care Unit

IMP - Investigational Medicinal Product

IRB - Institutional Review Board

mg - milligram

NHSN - National Healthcare Safety Network

PI - Principal Investigator

PK - Pharmacokinetics

PLEX - Polymer- Lipid Encapsulation matrix

POD - Post Operation Day

PVD - Peripheral Vascular Disease

QA - Quality Assurance

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 12 of 98

QC - Quality Control

SAR - Serious Adverse Reaction

SAE - Serious Adverse Event

SDV - Source Data Verification

SOC - Standard of Care

SOP - Standard Operating Procedure

SSI - Surgical Site Infection

SSWI - Superficial Sternal Wound Infection

SUSAR - Suspected Unexpected Serious Adverse Reaction

TCP - Tricalcium Phosphate

TIA - Transient Ischemic Attack

TX - Treatment

USP - United States Pharmacopeia

WOCBP - Women Of Child Bearing Potential

w/w - weight per weight

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 13 of 98

1 TRIAL PERSONNEL

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	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 14 of 98

2 SUMMARY

Study Number:	Study D-PLEX-302
Study Title:	A Phase III, Prospective, Multinational, Multicenter, Randomized, Parallel Controlled, Two arms, Single Blind, Study to Assess the Efficacy and Safety of D-PLEX Administered Concomitantly with the Standard of Care IV Prophylactic Antibiotic Treatment (SOC) vs. SOC only, in Prevention of Post-Cardiac Surgery Sternal Infections.
Phase:	Phase III
Objectives:	The primary objectives are as follows: - To assess D-PLEX efficacy in preventing sternal infections over a period of 90 days (3 months) post cardiac surgery with median sternotomy, in patients with high risk for infection compared to the control arm. - To assess D-PLEX safety
Number of Centers:	About [REDACTED] centers in US (about [REDACTED] sites), Europe and Israel will participate in this study.
Subject Population:	- Male and female, 18 years and older, Scheduled for elective and/or urgent median sternotomy in cardiac surgery. - Patient with the two following comorbidities: Diabetes Mellitus AND BMI≥30 OR Patients with one of the two comorbidities Diabetes Mellitus OR BMI≥30 AND One of the following comorbidities : Current/Previous smoking history ≥30 pack year OR Chronic Obstructive Pulmonary Disease (COPD). - Subjects who meet the eligibility criteria and provide signed informed consent will be enrolled in the study.
Number of Subjects:	Minimum of [REDACTED] subjects will be enrolled into the study, with a maximum of about [REDACTED] subjects. Number of subjects is determined following blinded non-comparative sample size adaptation: When [REDACTED] subjects completed 90 days (3 months) of primary end point.
Investigational Medicinal Product:	D-PLEX is a new formulation of extended release of Doxycycline (active ingredient). Each vial contains [REDACTED] total product, corresponding to [REDACTED] of doxycycline hyclate. The

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 15 of 98

	total percentage of free base doxycycline in D-PLEX is [REDACTED] which is equivalent to [REDACTED] doxycycline hyclate. D-PLEX is supplied as sterile powder to be reconstituted with saline and is intended for single administration. D-PLEX contain [REDACTED] and [REDACTED] non active ingredients that are [REDACTED]
Indication:	D-PLEX is indicated for prevention of post cardiac surgery sternal infection.
Other Drugs Required for the Study:	NA
Standard of Care (SOC):	The SOC prophylactic antibiotic treatment is based on <i>"The society of thoracic surgeons practice guideline series: antibiotic prophylaxis in cardiac surgery, part II: antibiotic choice."</i> The SOC prophylactic antibiotic treatment will be consistent and standardized for all sites in the clinical study.
Dose Administration:	D-PLEX dose is individualized, at least 2 vials and no more than 3 vials ([REDACTED] each) of D-PLEX. The total dose applied at the surgical site is determined based on sternum length, which is evaluated by the surgeon.
Duration of Dosing:	D-PLEX is administered as a single application. The active ingredient (doxycycline) is being continuously released for approximately 3-4 weeks.
Study Design:	Eligible subjects will be randomized to either the treatment arm (D-PLEX + SOC prophylactic antibiotic treatment) or to control arm (SOC prophylactic antibiotic treatment only), in a 1:1 ratio. <u>The SOC is based on:</u> The Society of Thoracic Surgeons practice guideline series: antibiotic prophylaxis in cardiac surgery, part II: antibiotic choice; 1. In patients for whom cefazolin is the appropriate prophylactic antibiotic for cardiac surgery, administration within 60 minutes of the skin incision is indicated. The pre-operative prophylactic dose of cefazolin for a patient of greater than 60 kg body weight is recommended to be 2g. 2. When surgical incision remains open in the operating room, to patients with normal renal function, a second dose should be administered every 3 to 4 hours.

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---------------------	---------------------------------

If it is apparent that cardiopulmonary bypass will be discontinued within 4 hours, it is appropriate to delay until perfusion is complete to maximize effective blood levels.

3. In patients for whom vancomycin is an appropriate antibiotic for cardiac surgery, a dose of [REDACTED] or a weight adjusted dose of [REDACTED] mg/kg administered intravenously slowly over 1 hour, with completion with 1 hour of the skin incision, is recommended. A second dose of vancomycin of [REDACTED] mg/kg may be considered during cardiopulmonary bypass, although its usefulness is not well established.
4. For patients who receive an aminoglycoside (usually gentamycin, [REDACTED] mg/kg) in addition to vancomycin before cardiac surgery, the initial dose should be administered within 1 hour of the skin incision. Redosing an aminoglycoside during cardiopulmonary bypass is not indicated and may be harmful.

The standard of care of IV prophylactic antibiotics will be consistent for all sites that will participate in the clinical study.

The duration of the antibiotic prophylaxis may be up to the course of 24 hours.

Subjects will be blinded to the study arm.

For subjects randomized to the investigational treatment arm, D-PLEX treatment will be applied at the end of the index surgery just before closing the chest, as an adjunct to the SOC prophylactic antibiotic treatment.

Pre- and post-operative care for both arms will be performed per site SOC. Post-operative resumption of activity is at the discretion of investigator based upon subject medical condition.

All adverse events (AE/SAE) including death will be collected and recorded throughout the study.

The planned clinical program will establish the efficacy and safety of D-PLEX by reduction in the number of sternal infections comparing to control arm observed during the first 90 days (3 months) along with demonstration of the safety profile.

Primary Efficacy Endpoint:

Infection rate as measured by the proportion of subjects with a sternal wound infection event within 90 days (3 months) post sternotomy for cardiac surgery.

Sternal infection is composed of Deep Sternal Wound Infection (DSWI) and Superficial Sternal Wound Infection (SSWI).

Mortality from any reason within 90 days (3 months) post sternotomy, will be analyzed as treatment failure.

Key Secondary Efficacy Endpoints:

- Average number of Hospitalization days post sternotomy, due to sternal surgical site infection.
- Average ASEPSIS assessment score during 90 days (3 month) post sternotomy.
- Number of surgical re-intervention due to sternal surgical site infections (including OR and non-OR/ bed-side, procedures)

Sternal infection is identified by the predefined criteria based on CDC/NHSN Procedure-associated Module SSI and Surveillance Definitions for specific types of infections (January 2018) definition.

'Other Secondary' Endpoints (recommended by regulators):

- Incidence of Superficial Sternal Wound Infections (SSWI) during 90 days (3 months) post sternotomy.
- Incidence of Deep Sternal Wound Infection (DSWI) during 90 days (3 months) post sternotomy.
- Mortality rate associated with Sternal Wound Infection (SWI) within 90 days (3 month) post sternotomy.
- Determination of susceptibility to Doxycycline of any organism recovered from a Sternal Surgical Site Infection.

Additional Secondary Efficacy Endpoints

- Overall number of hospitalization days, for any reason.
- Number of readmissions due to Sternal Surgical Site infection.
- Average number of Antibiotic Treatment (overall IV and other administration modes, e.g. oral) days due to Sternal Surgical Site infection (SSWI & DSWI).
- Average number of Antibiotic Treatment (IV) days due to Sternal Surgical Site infection (SSWI and DSWI).

	• Time to Sternal Surgical Site Infection (Post Operating Day) post sternotomy. • Average number of analgesic treatment days. • Pain VAS Score assessment. <u>Safety Evaluation:</u> The following safety parameters will be evaluated during the trial period (6 months): • Adverse events, physical examinations & vital signs. • Sternum stability, as indication for sternal bone union, will be clinically evaluated by an investigator that was not involved in the surgery, and is blinded to the treatment arm. In case of suspected instability at end of study visit (6 months after surgery), a chest CT (non-contrast) will be performed. • Surgical wound healing will be assessed by physical examination by an investigator that was not involved in the surgery, and is blinded to the treatment arm. A Modified Vancouver Scar Scale will be provided to the blinded investigators to prompt meticulous evaluation of surgical wound healing. • Safety laboratory parameters: Routine hematology & chemistry. Urinalysis will be collected on screening and thereafter at investigator discretion. <u>Pharmacokinetic Parameters</u> Thorough Pharmacokinetic Study for assessing systemic exposure of Doxycycline will be performed on a subset of trial participants at selected sites (total of approximately [REDACTED] subjects).
Inclusion Criteria:	Subjects who meet all of the following criteria will be eligible to participate in the study: 1. Subjects scheduled to elective and/or urgent median sternotomy for cardiac surgery, who are preoperative hemodynamically stable. 2. Males and females. 3. Subjects age of 18 and older. 4. Patient with the two following comorbidities: Diabetes Mellitus AND BMI$\geq$30 OR

	Patients with one of the two comorbidities Diabetes Mellitus OR BMI$\geq$30, AND One of the following comorbidities : Current/Previous smoking history $\geq$30 pack year OR Chronic Obstructive Pulmonary Disease (COPD). 5. Female of childbearing potential should have a negative serum pregnancy test prior to index procedure. <i>Note: All female of childbearing potential must agree to use a highly effective method of contraception (see Section 6.3 – CONTRACEPTIVE METHODS)</i> 6. Subject is willing and able to provide a signed Informed Consent Form and is willing and able to comply with study's procedures including follow-up visits.
Exclusion Criteria:	Subjects who meet any of the following exclusion criteria are prohibited from participating in the study: - Subjects undergoing partial sternotomy. - Subjects with any preoperative active significant infection. - Subjects that received oral or IV doxycycline during the last 4 weeks prior to screening. - Subjects with sensitivity to doxycycline and/or to tetracycline family of drugs and/or other IMP ingredients. - Subjects with known allergies to more than any 3 substances. (An allergy questionnaire will be filled during the screening process). - Subjects with history of allergic/hypersensitivity reaction to any substance having required hospitalization and/or treatment with intra-venous steroids/epinephrine or in the opinion of the investigator the patient is at high risk of developing severe allergic/hypersensitivity reactions. - Subjects with uncontrolled Asthma (GINA III-IV). - Subjects with chronic urticaria. - Immunocompromised subjects from any reason, at screening. - Subjects with renal failure requiring dialysis. - Subjects scheduled for major organ transplantation and/or to other significant concomitant surgical procedure.

	12. Subjects scheduled for mechanical assist device: left ventricular (LVAD) and/or right ventricular (RVAD), or artificial heart. 13. Subjects scheduled to be treated with preventive negative pressure devices. 14. Subjects undergone CVA/TIA within the past 3 months prior to randomization. 15. Subjects that have undergone previously, any cardiac surgery through sternotomy. 16. Subjects with active or previous malignancy in the chest area. 17. Any subject with active malignancy or with malignancy that has not been in complete remission for at least 5 years. Subjects who have had carcinoma <i>in situ</i> of the cervix, squamous cell carcinoma of the skin and basal cell carcinoma of the skin, are eligible. 18. Pregnant or breast-feeding women or women of childbearing age not protected by a highly effective contraceptive method of birth control (<i>see Section 6.3 – CONTRACEPTIVE METHODS</i>). 19. Subjects enrolled in any intervention study with an investigational medicinal product and/or received any investigational medicinal product within 30 days or 5½ half-lives of the product prior to enrollment (whichever is longer). 20. 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, medical condition or residency distanced from site that may jeopardize FU visits attendance etc.).
Interim for Efficacy and Futility Analysis	An interim analysis to evaluate efficacy and futility will be conducted after a total of N = [REDACTED] subjects (constitute approximately [REDACTED] of the minimal total sample size), have been assessed for primary efficacy (infection primary endpoint within 90 days) analysis. In case the sample size will be increased following blinded non-comparative sample size adaptation, the number of subjects included in the interim analysis will be increased accordingly to constitute approximately [REDACTED] of the updated total sample size. The futility stopping rule will be formulated using Conditional Power (CP) such that: If $CP < [REDACTED]$ then the study could be declared futile and terminated.
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	This stopping rule was chosen to spend [REDACTED] of the risk (Type II) at the interim out of the overall [REDACTED] in the study. The CP will be calculated assuming the design assumptions on the unobserved data. The efficacy stopping rule will be formulated by one sided p-value using the published Lan Demetz O'Brien Fleming error spending function such as if the one sided p-value < [REDACTED] (or two sided < [REDACTED]), then the study will be stopped for proven efficacy. The level of significance at the end of the study will be 0.046. The p-value will be calculated using Fisher's Exact test. All of the above calculations were performed using EAST software v6.4. The interim analysis will be conducted by an Independent Biostatistician (external to sponsor). Based on this analysis, the DMC will recommend for futility or proven efficacy, if the above rule is met and safety data will support it.
Sample Size Calculation:	<u>Sample Size and Power Calculations</u> Power calculation and determination of sample size is based on demonstrating superiority of D-PLEX to SOC on the primary endpoint of infection rate at up to 90 days post-surgery. It is assumed that population infection rates are [REDACTED] and [REDACTED] in SOC and D-PLEX respectively reflecting [REDACTED] reduction in the risk. In addition, a [REDACTED] death rate is estimated for both treatments and will be considered as failure (infection), thus yielding [REDACTED] and [REDACTED] infection rates for SOC and D-PLEX respectively. The following assumptions were used for the sample size: • Randomization will allocate patients to D-PLEX and SOC in a 1:1 ratio. • Infection rates of D-PLEX and SOC are [REDACTED] and [REDACTED] respectively, yielding a relative risk of [REDACTED] reflecting [REDACTED] effect reduction in the risk. • One interim analysis (IA) is expected when approximately [REDACTED] of the subjects complete 90 days (3 months). This IA will be designated to stop the study for futility or proven efficacy. • Experiment-wise two sided [REDACTED] • [REDACTED] lost to follow-up rate.

Under the above assumption, a sample size of [REDACTED] evaluable patients in total ([REDACTED] per group) will provide [REDACTED] power to demonstrate superiority of D-PLEX. The sample size is determined by Fisher's exact test.

Blinded Sample Size reassessment

Rationale: The blinded non-comparative sample size reassessment will be conducted in order to confirm if the assumption on the SOC infection rate is valid and the intended sample size is adequate.

Responsibility: The blinded study Biostatistician who is involved in the study design will perform the above blinded non-comparative sample size re-assessment. A formal report with the methodology and conclusion will be generated.

Timing: The blinded non-comparative sample size re-assessment will be performed when N = [REDACTED] subjects (constitute approximately [REDACTED] of the minimal total sample size), have been assessed for primary efficacy (primary outcome measure within 90 days) analysis.

Maximal Sample Size: The maximal sample size, predefined to upsize the study, will not exceed [REDACTED] subjects. This sample size was based on calculation of clinical meaningful effect prorated in acceptable infection rate.

Procedure for blinded non-comparative sample size adaptation:

Step 1: Estimate the SOC Infection Rate (IR) + Death Rate (DR) in a fully blinded manner using the assumption of the treatment effect from the design stage (i.e. [REDACTED] risk reduction). That is, given the pooled observed Infection Rate + Death Rate with the assumed [REDACTED] treatment effect, the SOC IR + DR will be extracted.

Step 2: Calculate the pooled observed lost to follow up.

Step 3: Using the above updated SOC IR+DR as well as the observed lost to follow-up, a new power calculation will be performed while keeping all other assumptions, which were used for the sample size calculation, similar as in the design stage (i.e. significance level, assumed treatment effect, method of analysis etc.).

Step 4: Decision Rules:

The following rules will guide the final sample size determination:

	• If the re-estimated SOC IR+DR $\geq$ [REDACTED] and the recalculated power $>$ [REDACTED], then no change in the sample size will be made; • If the re-estimated SOC IR+DR is between [REDACTED] and the recalculated power $<$ [REDACTED], then the sample size will be increased up to [REDACTED] subjects to reach [REDACTED] power. The rate of [REDACTED] corresponds to [REDACTED] subjects for [REDACTED] power if all other assumptions mentioned for the sample size calculations are the same; • If the re-estimated SOC IR + DR is $<$ [REDACTED] then the maximal sample size of 1600 subjects will be used; • The sponsor reserves the right not to change the original sample size of [REDACTED] subjects if the SOC infection rate is slightly less (up to [REDACTED] than the original [REDACTED]
Statistical Methods:	Randomization: A total of [REDACTED] subjects, will be randomized, using a 1:1 assignment ratio to treatment with either D-PLEX ([REDACTED] subjects) or SOC ([REDACTED] subjects). A dynamic randomization procedure will be performed using an interactive response technology (IRT) to balance the treatment groups within: • Surgery duration of > 4 hours – yes/no • Usage of double mammary artery bypass – yes/no • BMI > 40 – yes/no • Co-existing of the co-morbidities yes/No: ○ Yes = BMI ≥ 30 AND Diabetes Mellitus AND (COPD or Current/Previous smoking history ≥ 30 pack year). ○ No = Otherwise <u>Analysis Set</u> Intent to treat (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 received. <u>Safety Analysis set</u> 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 Intent to Treat (mITT) - The Modified Intent to Treat analysis set (mITT) 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 at study entry.

The per protocol (PP) analysis set will be the subset of mITT patients with no major protocol violations, with valid 90-day observations on the primary endpoint. "Major" violation will be defined as one having the potential to affect study primary outcome¹ and will be specified in the SAP.

Primary Efficacy Endpoint

The primary efficacy endpoint in this trial is, for each subject, dichotomously scored infection:

- Infection = 1 (Yes) = presence of sternal surgical site infection at up to 90 days post-surgery or all cause mortality.
- Infection = 0 (No) = Otherwise.

Primary Efficacy Analysis

Primary analysis will be done on the ITT analysis set. The null hypothesis and alternative are as follows:

H0: Infection Rate_{D-PLEX} = Infection Rate_{SOC}

H1: Infection Rate_{D-PLEX} ≠ Infection Rate_{SOC}

The primary end-point of presence or non-presence of sternal surgical site infection up to 90 days post-surgery will be analyzed using Fisher's exact test with two-sided Alpha= 0.046 (due to interim analysis for efficacy).

The heterogeneity of treatment effects across sites will be explored by separately testing the treatment by site interaction using proc logistic with STRATA and EXACT options. This test will be performed in an exploratory fashion, and at a nominal significance level of [REDACTED]

Key Secondary Efficacy Endpoints and Analyses

The following key secondary analyses, involving the key secondary endpoints, will be tested according to the following hierarchical order used to control for multiple testing:

¹ FDA (CDER, CBER). Guidance for industry: E9 statistical considerations for clinical trials. 1998. p. 28.

1. The average number of Hospitalization days post sternotomy due to sternal surgical site infection will be analyzed using Poisson regression.
2. Average ASEPSIS assessments score during 90 days will be analyzed using Analysis of Variance (ANOVA).
3. The number of surgical re-interventions due to Sternal Surgical Site infection will be analyzed using poisson regression.

'Other Secondary' Efficacy Endpoints and Analyses (recommended by regulators):

The following other secondary endpoints will be analyzed in a descriptive manner. No inferential statistics will be conducted and p-values may be provided nominally for trends detection.

- Incidence of Superficial Sternal Wound Infections (SSWI) during 90 days post sternotomy will be analyzed using Fisher's Exact test similarly to the primary end-point.
- Incidence of Deep Sternal Wound Infections (DSWI) during 90 days post sternotomy will be analyzed using Fisher's Exact test similarly to the primary end-point.
- Mortality rate associated with Sternal Wound Infection (SWI) within 90 days (3 months) post sternotomy will be analyzed using Fisher's Exact test similarly to the primary end-point.
- Proportion of patients with susceptibility to Doxycycline of any organisms recovered from a Sternal Surgical Site Infection Site will be analyzed using logistic regression.

Further details on each analysis will be provided in a Statistical Analysis Plan signed and approved prior to data base lock.

Additional Secondary Efficacy Endpoints:

The following additional efficacy endpoints will be analyzed in an exploratory manner. No inferential statistics will be conducted and p-values may be provided nominally for trends detection.

- The analysis of the overall number of hospitalization days due to any reason will be based on the outcome of a contrast (D-PLEX+SOC vs. SOC) derived from Poisson Regression (SAS® PROC GENMOD with DIST=POISSON).
- The analysis of the number of readmissions due to Sternal Surgical Site infection will be based on the outcome of a contrast (D-PLEX+SOC vs. SOC) derived

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 26 of 98

	from Poisson Regression (SAS® PROC GENMOD with DIST=POISSON). • Number of days with Antibiotic Treatment (overall IV and other administration modes, e.g. oral) due to Sternal Surgical Site infection; SSWI and DSWI will be analyzed using Analysis of Covariance (ANCOVA). • Number of days with Antibiotic Treatment (IV) due to Sternal Surgical Site infection (SSWI and DSWI) will be analyzed using Analysis of Covariance (ANCOVA). • Time to Sternal Surgical Site Infection (Post Operating Day) post sternotomy will be analyzed using log rank test. Kaplan Meier figure will be created. • Number of days with analgesic treatment will be analyzed using Analysis of Covariance (ANCOVA). • Pain VAS Score assessment will be analyzed using Analysis of Covariance (ANCOVA).
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CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 27 of 98

3 INTRODUCTION

3.1 BACKGROUND

3.1.1 BACKGROUND INFORMATION ON THE PRODUCT

3.1.1.1 Product name

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

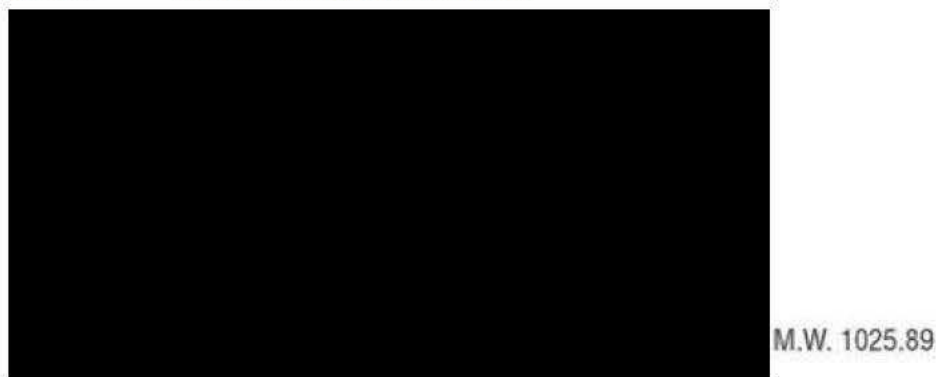
g D-PLEX per vial contains doxycycline hyclate equivalent to (mg) doxycycline free base.

3.1.1.2 Drug Substance Chemical Name and Structure

Doxycycline Hyclate

The chemical name of Doxycycline Hyclate is (dimethylamino)-

Figure 1: Structure of Doxycycline Hyclate



3.1.1.3 Proposed Indication

D-PLEX is indicated for prevention of post cardiac surgery sternal infection.

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

Dosage form: D-PLEX is a sterile biodegradable extended-release powder formulation of doxycycline intended for single use and supplied in vial presentations. Each doxycycline hyclate (doxycycline free base) in of D-PLEX formulation. The powder is reconstituted with sterile saline to form a paste immediately before application.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 28 of 98

Reconstitution: Immediately before application, the entire content of D-PLEX vials (2 or 3 vials) is emptied into a sterile mixing bowl. The reconstitution is then performed as follows (according to required dose): two vials are reconstituted with █ mL sterile saline solution, or, three vials are reconstituted with █ mL sterile saline solution to form a paste that is applied directly within the surgical site (i.e., on the sternum).

Note: The sterile saline is commercially available and will not be supplied by PolyPid.

Route of administration: Direct application of D-PLEX reconstituted paste in between and above the two halves of the sternum, immediately after reconstitution during the process of sternum closure at the completion of the surgical procedure. No D-PLEX is to be applied on the skin suture line.

Dosing regimen: D-PLEX is administered prior to surgical wound closure following open chest surgical procedures. The total dose applied at the surgical site is determined based on sternum length, which is evaluated by the surgeon. To provide adequate coverage of the surgical site, a minimum of two vials (█ for a sternum ≤ 22 cm) and a maximum of three vials (█ for a sternum > 22 cm) may be administered as per the table below. Each patient will either receive two vials of D-PLEX or three vials of D-PLEX; no patient will receive a fraction of a vial. Therefore, the total amount of Doxycycline base for a single surgical procedure with D-PLEX may be up to █ mg, which is below the maximum labelled oral (systemic) daily dose.

The individualized dose will follow the following scheme:

Sternum Length (cm)	# of vials to apply
≤ 22	2
> 22	3

3.1.1.5 D-PLEX Product

D-PLEX contain biodegradable and biocompatible non active ingredients that are TCP, polymers and lipids. D-PLEX is supplied in a glass vial (█/vial) as a sterile powder to be reconstituted to paste and is intended for a single administration.

3.1.1.6 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.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 29 of 98

3.1.2 UNMET NEED FOR STERNAL INFECTION PREVENTION FOLLOWING CARDIAC SURGERY

Sternal infections post-cardiac surgery are a major cause of post-operative illnesses resulting in increased morbidity and mortality.

In North America and Europe, approximately two million cardiac surgeries are performed annually (circa 5,000 are conducted in Israel). Cardiac procedures includes coronary artery bypass grafting (CABG) procedures, valve replacement, and repair, placement of temporary or permanent implantable cardiac devices, including ventricular assist devices (VADs) and ascending aorta surgeries associated with cardiac procedures.

One of the most deleterious complication following cardiac surgery is deep sternal wound infection (DSWI; mediastinitis). The incidence of deep sternal wound infections ranges between 0.3-3.2% (Kubota et al. 2013, Sjogren et al. 2006, van Diepen et al. 2014, Beckmann et al. 2011, Simek et al. 2013, Juhl et al. 2012) with mortality rate as high as 40% (Kubota et al. 2013). In high-risk patients undergoing cardiac surgery, DSWI rates are even-higher (5-7%, Grauhan et al. 2013, Bennett-Guerrero et al. 2010). Prolonged hospitalization of these patients can last weeks or months, often with repeated surgical interventions required and long-term antibiotic treatment in cases of osteomyelitis. Superficial sternal wound infection (SSWI) is also a common complication of cardiac surgery, though more reliably treated with systemic and/or topical antibiotics than deep wound infections. However, even superficial wound infections can lead to clinically significant complications such as non-healing wounds, systemic infections and surgical site revision surgeries. If hospitalization is prolonged, there is an increased risk of developing co-infection with antimicrobial-resistant pathogens.

The overall incidence of DSWI and SSWI in patients with diabetic and/or with body mass index >30 was reported to be 8.7% in a study reported in 2010 in the United States (U.S.) despite prophylactic antibiotic treatment (Bennett-Guerrero et al. 2010).

Though the risk of surgical site infections (SSIs; including mediastinitis and sternal wound infection) is relatively low, the consequences of infection are severe (Bratzler et al. 2013). The current standard of care for preventing SSIs does not reliably avoid all serious and potentially fatal infectious outcomes; therefore, the prevention of post cardiac surgery sternal infections represents an unmet medical need.

3.1.3 JUSTIFICATION FOR THE USE OF D-PLEX FOR PREVENTION OF SSI FOLLOWING CARDIAC PROCEDURES

D-PLEX represents a novel formulation of doxycycline that improves the potential for prevention of post cardiac surgery sternal infections over currently utilized preventive measures. Such measures include:

- Application of mechanical measures (e.g. pre-operative bathing and hair removal)
- A pre-incisional dose of cefazolin or cefuroxime with appropriate intraoperative re-dosing (e.g., use of lavage fluids containing antibiotics)

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 30 of 98

- Introduction of a sterile area in the operating room in order to restrict movement, and increased sterile airflow and air exchange in the proximity of the patient
- Wearing of specialized gowning by surgeons ("space suits") to prevent airflow from the surgeon or operating room in the direction of the patient.

While these measures have reduced the rate of infection, they have not completely eliminated it. Part of the reason for the persistence of these types of infections (despite the above procedures) is local hematoma forms at the surgical site following surgery. This is a wet environment that encourages 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 lymph fluid at the hematoma site are disrupted. Therefore, systemic antibiotics and immune defenses have impaired ability to reach the affected areas, causing these areas to be more susceptible to proliferation of bacteria and subsequent development of infection. With low local prophylactic antibiotic concentrations, there is a risk of developing antibiotic resistant bacteria, raising the systemic antibiotic dose may cause systemic toxicity. To overcome this problem, some surgeons locally dispense antibiotics before closing of the incision. This antibiotic treatment has shown to be effective for a very short duration of one to two hours and does not prevent development of bacterial infection (Schersten 2007, Eklund et al. 2005, Vander Salm et al. 1989).

For example, the use of currently approved topical antimicrobials, mainly gentamicin or vancomycin, applied to the sternum during cardiac procedures in combination with IV antibiotics to prevent mediastinitis have not been clearly established (Schersten 2007, Eklund et al. 2005, Vander Salm et al. 1989). Gentamicin collagen sponges failed to show any efficacy in a large prospective, randomized controlled study of cardiac surgery in the U.S. (Bennett-Guerrero et al. 2010). Thus, alternative locally applied antibiotics or antibiotic-containing products have not proved effective. A product such as D-PLEX that can provide sustained locally high levels of doxycycline has the potential to achieve a level of infection prevention not achievable with currently approved products or procedures.

As indicated above, D-PLEX is a new formulation of extended-release of doxycycline for administration in the sternal surgical site: in between and above the two halves of the sternum following median sternotomy performed in cardiac procedures. D-PLEX releases doxycycline immediately upon product administration and during approximately 3-4 weeks following administration, which is the time period in which most of the sternal infections occur (Jonkers et al. 2003).

3.2 NON-CLINICAL STUDIES

Non-clinical pharmacology studies, performed to demonstrate anti-bacterial activity of D-PLEX in prevention of infections in bone and soft tissue environment in human, included:

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 31 of 98

- Six pivotal studies in rabbit sternal wound infection model (each with a different representative strains of clinically relevant bacteria²) 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 hematology parameters and the reduction in bacteria count isolated from the sternum wound (i.e. bone defect and surrounding soft tissue) and infection-related toxicities.
- Pivotal efficacy dose response studies in rabbits using D-PLEX with different doxycycline concentrations against 2 different inoculum concentrations of MRSA followed a dose-response trend which indicates that the chosen doxycycline concentration in D-PLEX is the most effective in decreasing the level of infection in a sternal defect model.
- 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).

Non-clinical pharmacokinetic studies performed to demonstrate doxycycline local and systemic concentration after its administration, showed:

- Concentrations of unbound doxycycline higher than [REDACTED] for 72 hours, and higher than [REDACTED] µg/mL for up to 30 days could be detected at the administration site in rabbit sternal model (except for one time point at 21 days, where a concentration of [REDACTED] µg/mL was observed). These levels exceed the MIC values of sensitive bacterial strains for a prolonged period of time. The levels measured during the first few days also exceed the MIC values of many resistant bacteria.
- D-PLEX ability to release doxycycline over a prolonged period of time in rats after Subcutaneous (SC) administration of D-PLEX.

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

- A safety pivotal study showed that D-PLEX at an applied maximal feasible dose in rabbit sternal wound model, comparable on a mg/kg basis to twice the maximum doxycycline free base in D-PLEX applied dose to a 70-kg adult (equivalent to [REDACTED] mg doxycycline free base in [REDACTED] vials of D-PLEX), demonstrates no systemic effects, no deleterious effects on healing of the sternal site and no effect on strength of the sternal bone during 15 months after administration.
- A safety study conducted in rats did not show any adverse effect related to D-PLEX when administered between the sternal bone and the overlying muscles in a dose comparable to [REDACTED] D-PLEX in a 70-kg adult on a mg/kg basis during 3 months after administration.

² i.e. *Methicillin Resistant Staphylococcus Aureus (MRSA)*, *Staphylococcus epidermitis coagulase negative (CoN)*, *Enterococcus faecalis*, *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 32 of 98

- 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 (which is very unlikely to happen and hence represents a worst-case scenario) showed that D-PLEX exposure (comparable on mg/kg basis to [REDACTED] gr 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.

3.3 CLINICAL DATA

A phase 1b/2 clinical study, conducted in Israel, included two parts: Part 1, Open Label, Single Arm and Part 2, Randomized Controlled, Single Blind Study, to assess Safety and Efficacy of D-PLEX concomitantly with Standard of Care (SOC) vs. Standard of Care alone in the prevention of sternal infection post cardiac surgery for up to 24 weeks following surgery. The study included a total of [REDACTED] subjects that were scheduled for open heart surgery, including but not limited to subjects with high-risk factors for infection : 20 subjects in part 1 (all in the treatment arm) and [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 randomized to D-PLEX with SOC group. One subject withdrew consent before treatment.

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

- A single patient in the SOC group ([REDACTED]) experienced a superficial sternal wound infection (SSWI) on Day 6 post-surgery.
- No subjects in the treatment group experienced any sternal wound infection ([REDACTED]).
- No hospitalizations, readmissions or surgical re-interventions 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] subjects in the SOC group ([REDACTED]) and [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 D- PLEX or the study procedure.
- PK data demonstrated that D-PLEX releases doxycycline continuously over a period of 30 days as well as that the systemic exposure of D-PLEX is lower than any dose of doxycycline based product administered orally; related to Cmax and AUC0-t compared to ACTICLATE, VIBRAMYCIN and DORYX (labeling information data) and a medical literature citation (Gschwend, 2007).

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 33 of 98

3.3.1 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 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 1b/2 clinical study, which demonstrated lower systemic exposure of doxycycline compared to approved orally doxycycline formulations.

3.4 POTENTIAL BENEFITS OF D-PLEX FOR PREVENTION OF POST CARDIAC SURGERY STERNAL INFECTIONS

- Prevention of sternal infection, which may occur within 90 days (3 months) post median sternotomy.
- Significant improvement in prevention of sternal infection when used in higher risk population (Diabetes Mellitus, BMI $\geq$ 30, Chronic Obstructive Pulmonary Disease (COPD), Current/Previous smoking history $\geq$ 30 pack year).
- Significant improvement in prevention of sternal infection when used in surgical procedures that hold high risk of infection such as: median sternotomy for coronary artery bypass grafting, internal mammary artery harvesting etc.
- Induces faster recovery and early return to normal activity as compared to SOC post median sternotomy.
- Reduction in the number of surgical re-interventions due to sternal wound infections.
- Reduction in the number of hospitalizations and/ or shortening hospitalization, thus providing financial savings and reducing hospital load.
- Potential for reducing comorbidities in vulnerable patients with sternal infection.
- Used concomitantly with SOC.
- Ease of administration, no special training is required.
- Broad spectrum activity of doxycycline.
- Local administration of effective dose of doxycycline during median sternotomy for cardiac 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.
- Low potential risk for development of bacterial resistance.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 34 of 98

- 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 and/or blood circulation and are biodegradable.

3.4.1 POTENTIAL RISKS OF D-PLEX FOR PREVENTION OF POST CARDIAC SURGERY STERNAL INFECTION AND THEIR MITIGATIONS

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

Mitigations:

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

b) 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 (substances).
- In case of severe allergic reaction to doxycycline, subject will be treated conservatively, in case needed the D-PLEX will be removed by the surgeon.

c) Potential of bacteria resistant to doxycycline-treatment failure/exacerbation of infection.

Mitigation:

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

d) 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.

e) Photosensitivity reaction: exaggerated sun burn reaction.

Mitigations:

- Close follow up of patients.

f) Transient hypercalcemia

Mitigation:

- Close follow up of patients.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 35 of 98

3.5 SAFETY & EFFICACY SUMMARY

Based on the current data available from the one clinical study with D-PLEX, in which 60 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, clinical trial with D-PLEX in patients undergoing median sternotomy for cardiac surgery may be beneficial.

4 OBJECTIVES

- To assess D-PLEX efficacy in preventing sternal infections over a period of 90 days (3 months) post median sternotomy for cardiac surgery, in patients with high risk for infection compared to the control arm.
- To evaluate D-PLEX safety over a period of 6 months.

5 EFFICACY & SAFETY ENDPOINTS

5.1 EFFICACY ENDPOINTS

The following will be evaluated in this trial:

Primary Efficacy Endpoint:

Infection rate as measured by the proportion of subjects with a sternal wound infection event within 90 days (3 months) post sternotomy for cardiac surgery.

Sternal infection is composed of Deep Sternal Wound Infection (DSWI) and Superficial Sternal Wound Infection (SSWI).

Mortality from any reason within 90 days (3 months) post sternotomy, will be analyzed as treatment failure.

Key Secondary Efficacy Endpoints:

- Average number of Hospitalization days post sternotomy, due to sternal infection.
- Average ASEPIS assessment score during 90 days (3 month) post sternotomy.
- Number of surgical re-intervention due to sternal surgical site infections (including OR and non-OR/bed-side, procedures)

Sternal infection is identified by the predefined criteria based on CDC/NHSN Procedure-associated Module SSI and Surveillance Definitions for specific types of infections (January 2018) definition.

'Other Secondary' Endpoints (requested by regulators):

- Incidence of Superficial Sternal Wound Infections (SSWI) during 90 days (3 months) post sternotomy.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 36 of 98

- Incidence of Deep Sternal Wound Infection (DSWI) during 90 days (3 months) post sternotomy.
- Mortality rate associated with Sternal Wound Infection (SWI) within 90 days (3 month) post sternotomy.
- Determination of susceptibility to Doxycycline of any organism recovered from a Sternal Surgical Site Infection.

Additional Secondary Efficacy Endpoints

- Overall number of hospitalization days, for any reason.
- Number of readmissions due to Sternal Surgical Site infection.
- Average number of Antibiotic Treatment (overall IV and other administration modes, e.g. oral) days due to Sternal Surgical Site infection (SSWI & DSWI).
- Average number of Antibiotic Treatment (IV) days due to Sternal Surgical Site infection (SSWI and DSWI).
- Time to sternal wound infection (Post Operating Day) post sternotomy.
- Average number of analgesic treatment days.
- Pain VAS Score assessment.

5.2 SAFETY ENDPOINTS

The following safety parameters will be evaluated during the trial period (6 months):

- Adverse events, physical examinations & vital signs.
- Sternum stability, as indication for sternal bone union, will be clinically evaluated by an investigator that was not involved in the surgery, and is blinded to the treatment arm. In case of suspected instability at end of study visit (6 months after surgery), a chest CT (non-contrast) will be performed.
- Surgical wound healing will be assessed by physical examination by an investigator, that was not involved in the surgery, and is blinded to the treatment arm. A modified Vancouver Scar Scale will be provided to the blinded investigators to prompt meticulous evaluation of surgical wound healing.
- Safety laboratory parameters: Routine hematology & chemistry.
Urinalysis will be collected on screening and thereafter at investigator discretion.

Pharmacokinetic Parameters

Thorough Pharmacokinetic Study for assessing systemic exposure of Doxycycline will be performed on a subset of trial participants at selected sites (total of approximately [REDACTED] subjects).

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 37 of 98

5.3 TRIAL DESIGN

This is a Phase III, Prospective, Multinational, Multicenter, Randomized, Parallel Controlled, Two arms, Single Blind, Study to Assess the Efficacy and Safety of D-PLEX Administered Concomitantly with the Standard of Care IV Prophylactic Antibiotic Treatment (SOC) vs. SOC only, in Prevention of Post-Cardiac Surgery Sternal Infections.

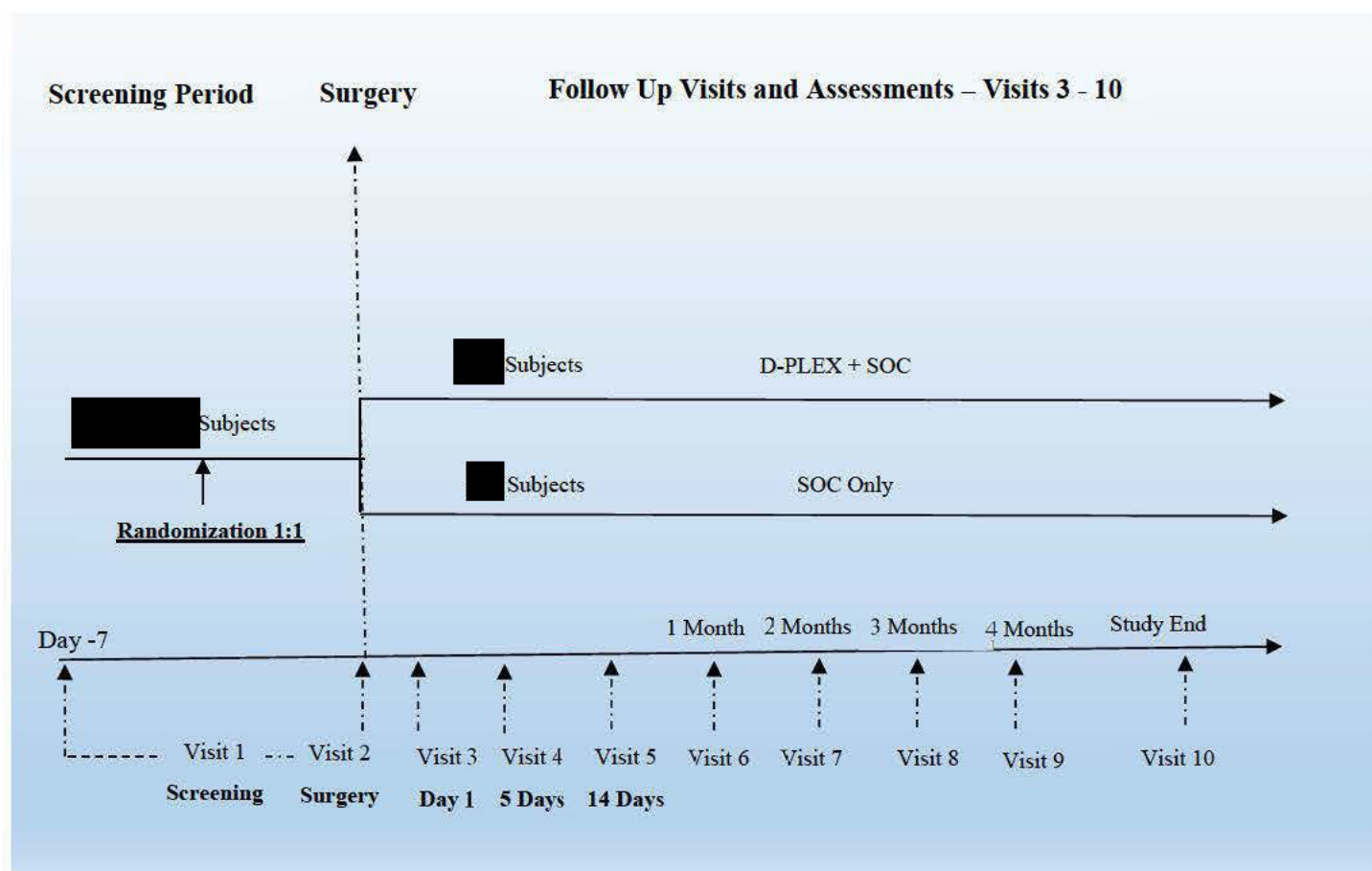
5.3.1 OVERALL TRIAL DESIGN

Males and females, 18 years and older, Scheduled to elective and/or urgent median sternotomy in cardiac surgery with both Diabetes Mellitus and BMI $\geq$ 30.

OR Diabetes Mellitus/BMI $\geq$ 30 AND at least one additional risk factor of the following:

- Current/previous smoking history $\geq$ 30 pack year.
- Chronic Obstructive Pulmonary Disease (COPD).

Subjects who meet the eligibility criteria, are willing and able to provide an Informed Consent Form and are willing and able to comply with study's procedures including follow-up visits, will be enrolled into the study and will be treated with D-PLEX concomitantly with SOC IV prophylactic antibiotic treatment vs. SOC IV prophylactic antibiotic treatment only.



CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 38 of 98

D-PLEX will be applied at the end of the index surgery just before closing the chest, as an adjunct to the standard care of IV prophylactic antibiotic treatment, immediately prior to sternal and tissue closure. Sternum and chest closure will be performed per investigator discretion.

Pre- and post-operative care will be performed per site's SOC. Post-operative resumption of activity are at the discretion of investigator based on subject medical condition.

Spontaneous adverse events (AEs) including any death, will be recorded throughout the study.

The study will assess the efficacy and safety of D-PLEX by preventing sternal infection post median sternotomy for cardiac surgery, in patients with high risk for infection, compared to the control arm.

Additional follow up for prolonged safety assessments only, will be done until 6 months post-surgery.

5.4 DESCRIPTION OF THE DURATION OF TREATMENT, SUBJECT PARTICIPATION AND TRIAL FOLLOW-UP

A number of factors have been considered for determining the 6 months follow up period.

Primarily, allowing a sufficient follow up period to demonstrate the initial and sustainable (prolonged) efficacy and safety endpoints of D-PLEX including the safety and efficacy of doxycycline incorporated into D-PLEX.

The anti-infective effect of D-PLEX will be assessed by preventing sternal infection post median sternotomy for cardiac surgery over a period of 90 days (3 months) post operation. The prevention of Infection rate is measured by the reduction in the number of subjects with sternal infections. Six (6) months period is a sufficient time to demonstrate the prolonged effect.

6 SELECTION OF SUBJECTS

It is anticipated that a total of [REDACTED] evaluable 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 scheduled to elective and/or urgent median sternotomy for cardiac surgery, who are preoperative hemodynamically stable.
2. Males and females.
3. Subjects age of 18 and older.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 39 of 98

4. Patient with the two following comorbidities:

Diabetes Mellitus AND BMI $\geq$ 30

OR

Patients with one of the two comorbidities:

Diabetes Mellitus OR BMI $\geq$ 30

AND

One of the following comorbidities:

Current/Previous smoking history $\geq$ 30 pack year OR Chronic Obstructive Pulmonary Disease (COPD).

Please ensure potential subject's comorbidities meet <u>at least</u> one of the below combinations		
Option 1	Diabetes Mellitus	BMI $\geq$ 30
Option 2	BMI $\geq$ 30	Current/Previous smoking history $\geq$ 30 pack year
Option 3	BMI $\geq$ 30	Chronic Obstructive Pulmonary Disease (COPD)
Option 4	Diabetes Mellitus	Current/Previous smoking history $\geq$ 30 pack year
Option 5	Diabetes Mellitus	Chronic Obstructive Pulmonary Disease (COPD)

5. 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 (see Section 6.3 – CONTRACEPTIVE METHODS)

6. Subject is willing and able to provide a signed Informed Consent Form and is willing and able to comply with study's procedures including follow-up visits.

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.

- Subjects undergoing partial sternotomy.
- Subjects with any preoperative active significant infection.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 40 of 98

3. Subjects that received oral or IV doxycycline during the last 4 weeks prior to screening.
4. Subjects with sensitivity to doxycycline and/or to tetracycline family of drugs and/or other IMP ingredients.
5. Subjects with known allergies to more than any 3 substances. (An allergy questionnaire will be filled during the screening process).
6. Subjects with history of allergic/hypersensitivity reaction to any substance having required hospitalization and/or treatment with intra-venous steroids/epinephrine or in the opinion of the investigator the patient is at high risk of developing severe allergic/hypersensitivity reactions.
7. Subjects with uncontrolled Asthma (GINA III-IV).
8. Subjects with chronic urticaria.
9. Immunocompromised subjects from any reason, at screening.
10. Subjects with renal failure requiring dialysis.
11. Subjects scheduled to major organ transplantation and/or to other significant concomitant surgical procedure.
12. Subjects scheduled for mechanical assist device: left ventricular (LVAD) and/or right ventricular (RVAD), or artificial heart.
13. Subjects scheduled to be treated with preventive negative pressure devices.
14. Subjects undergone CVA/TIA within the past 3 months prior to randomization.
15. Subjects that have undergone previously, any cardiac surgery through sternotomy.
16. Subjects with active or previous malignancy in the chest area.
17. Any subject with active malignancy or with malignancy that has not been in complete remission for at least 5 years. Subjects who have had carcinoma in situ of the cervix, squamous cell carcinoma of the skin and basal cell carcinoma of the skin, are eligible.
18. Pregnant or breast-feeding women or women of childbearing age not protected by a highly effective contraceptive method of birth control (*see section 6.3 – CONTRACEPTIVE METHODS*)).
19. Subjects enrolled in any intervention study with an investigational medicinal product and/or received any investigational medicinal product within 30 days or 5½ half-lives of the product prior to enrollment (whichever is longer).
20. 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, medical condition or residency distanced from site that may jeopardize FU visits attendance etc.).

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 41 of 98

6.3 CONTRACEPTIVE METHODS

Women of child bearing potential (WOCBP), will only be included in a clinical study after confirmed menstrual period and a negative serum pregnancy test.

All WOCBP must agree to use a contraception method from the following highly effective contraception methods for the duration of the study:

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

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

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

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

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

Note: The following are not acceptable methods of contraception:

- Periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea method (LAM).
- Male condom with cap, diaphragm, or sponge with spermicide.
- Male and female condom should not be used together (due to risk of failure with friction).

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 42 of 98

7 RECRUITMENT

Patient recruitment will be conducted in cardiac surgical admission units at sites in US, Europe & Israel.

8 STUDY PROCEDURES

8.1 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. 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 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, blood test etc.) 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 in accordance with IRB/EC approval. If relevant, all affected subjects shall be asked to confirm their continuing informed consent in writing.

8.2 SCREEN FAILURE

Subject who signed the informed consent and did not meet the eligibility criteria and was not randomized will be considered as screen failure. For such subject the reason for screen failure will be recorded in the screening log, the CRF and in the source documents.

8.3 RANDOMIZATION

Upon confirmation that the subject fulfils all inclusion criteria and no exclusion criterion is met, subject will be randomized through an interactive web randomization system (IWRS) integrated with the eCRF. A dynamic randomization procedure will be performed to balance the treatment groups based on subject's information collected during screening period.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 43 of 98

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

- Surgery duration of > 4 hours – yes/no
- Usage of double mammary artery bypass – yes/no
- BMI > 40 – yes/no
- Co-existing of three co-morbidities: BMI $\geq$ 30 AND Diabetes Mellitus AND (COPD or Current/Previous smoking history $\geq$ 30 pack year)
 - Yes = BMI $\geq$ 30 AND Diabetes Mellitus AND (COPD or Current/Previous smoking history $\geq$ 30 pack year)
 - No = Otherwise

Randomization will be done using dynamic randomization which will assign subjects in a 1:1 ratio to:

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

Instructions and training for randomization process using the IWRS system will be provided during site initiation visit.

8.4 SUBJECT ENROLLMENT

Once a subject is randomized to either one of the study arms (treatment or control arm), the subject will be considered as enrolled to the study.

8.5 BLINDING

This is a single blind clinical trial. In order to protect the integrity of the study, the study will be treated as if it were a blinded study in all ways other than the physician having knowledge of the treatment assignment, until the study is completed and the data sets are cleaned and locked.

In this study there will be two treatment arms: D-PLEX Administered Concomitantly with the Standard of Care (SOC) and SOC only. Two investigators will be involved in the treatment and assessment of the study outcome measures of the subject. Surgeon investigator will perform the surgery and administer the study drug. This investigator will be unblinded to the treatment arm. Second investigator will assess the study outcomes and will be blinded to the treatment arm.

Subjects will be blinded to the treatment arm assignment. The unblinded study site personnel will not disclose the treatment arm to the blinded investigator, to the subject, his/her family, to health care providers outside of the surgery department and to the study sponsor.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 44 of 98

8.5.1 EMERGENCY UNBLINDING

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

As a general rule, randomization 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 surgery and all the staff attending the operating room are expected to know the treatment arm of the subject. If unblinding is deemed necessary, the investigator can access information regarding the treatment arm information.

The Investigator is requested to maintain the blinding as much as possible. Investigators are encouraged to discuss with the sponsor Medical Monitor 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 on the corresponding CRF (case report form) page.

8.6 END POINT - ASSESSMENT of STERNUM INFECTION (AFTER SURGERY, THROUGH THE STUDY)

Sternal infection is composed of Deep Sternal Wound Infection (DSWI) and Superficial Sternal Wound Infection (SSWI). Sternal infection will be identified by the following predefined criteria based on modification of the CDC's Criteria for Surgical Site Infection (SSI) & Surveillance Definition for Specific Types of Infections Chapters, January 2018 (see 9.7.1 & 9.7.2).

8.6.1 DEEP STERNAL WOUND INFECTION (DSWI)

CDC/NHSN SURVEILLANCE DEFINITIONS FOR SPECIFIC TYPES OF INFECTIONS (JANUARY 2018, p. 17-14)

The patient has at least one of the following:

1. Patient has organisms identified from mediastinal tissue or fluid by a culture or non-culture based microbiologic testing method, which is performed for purposes of clinical diagnosis or treatment [e.g., not Active Surveillance Culture/Testing (ASC/AST)].
2. Patient has evidence of mediastinitis on gross anatomic or histopathologic exam.
3. Patient has at least one of the following signs or symptoms: fever ($>38.0^{\circ}\text{C}$), chest pain*, or sternal instability*; and at least one of the following:
 - i. Purulent drainage from mediastinal area
 - ii. Mediastinal widening on imaging test

** with no other recognized cause*

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 45 of 98

8.6.2 SUPERFICIAL STERNAL WOUND INFECTION (SSWI)

SUPERFICIAL INCISIONAL PRIMARY (SIP) SSI (BASED ON PROCEDURE- ASSOCIATED MODULE SSI, JANUARY 2018, p. 9-8):

Date of event for infection occurs within 90 days after any NHSN operative procedure (where day 1 = the procedure date)

AND

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

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

AND

the patient has at least one of the following signs or symptoms: pain or tenderness; localized swelling; erythema; or heat.

- d. Diagnosis of a superficial incisional SSI by the surgeon or attending physician** or other designee.

*** The term attending physician for the purposes of application of the NHSN SSI criteria may be interpreted to mean the surgeon(s), infectious disease specialist, other physician on the case, emergency physician or physician's designee (nurse practitioner or physician's assistant).*

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

- Diagnosis/treatment of cellulitis (redness/warmth/swelling), by itself, does not meet criterion "d" for superficial incisional SSI. Conversely, an incision that is draining or that has organisms identified by culture or non-culture based testing is not considered a cellulitis.
- A stitch abscess alone (minimal inflammation and discharge confined to the points of suture penetration).

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 46 of 98

8.6.3 PROCEDURES IN CASE OF SUSPECTED STERNAL INFECTION

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

In Case OF	Action	Time Line
Suspected Sternal Infection (DSWI and/or SSWI)	Report any sternal infection suspicion through eCRF page "Sternal Infection"	within 24h of knowledge
	Collect Bacteriological Sample – BEFORE antibiotic treatment initiation (instructions for collections and shipment will be provided). Surgical wound healing will be assessed for signs of infection, by an investigator, that was not involved in the surgery, and is blinded to the treatment arm	Once suspicion arises
	Collect Hematology/Chemistry Blood Samples for Central Laboratory (as defined in the table of study procedures)	Immediately
	Ship Bacteriological and Blood Samples to Relevant Central Laboratory	Immediately
In case of suspected DSWI	Perform a clinical assessment of sternum stability (by an Investigator blinded to treatment arm). In case of suspected sternum DSWI with/without sternum instability, and pending patient clinical status and feasibility of mobilizing patient to imaging, perform a chest CT.	Once suspicion arises
	Send chest CT to blinded central CT reading core lab. Instructions for performing the chest CT scan and transfer of scan to central CT reading core lab, will be provided.	Immediately
	Blinded Investigator assessor will assess all the collected information and will confirm or exclude event of sternal infection.	As soon as possible
	Send a detailed email to medical monitor/study group email.	Within 5 working days
	Update sternal infection assessments and conclusion through eCRF page "Sternal Infection". Provide detailed information on confirmed sternal infection (re: treatment, outcome etc.)	As soon as possible

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 47 of 98

All collected information and investigator assessment of the suspected sternal infection will be forwarded to the blinded “End-points Adjudication Committee”.

“End-points adjudication committee” will review all collected information and will confirm or exclude sternal infection.

In case of dispute regarding sternal infection confirmation, “End-Point Adjudication Committee” adjudication will prevail.

All Sternal Infections will be collected and recorded on the “Sternal Infection” eCRF page.

8.7 SAFETY EVALUATION

Safety Evaluation:

The following safety parameters will be evaluated during the trial period (6 months):

- Adverse events, physical examinations & vital signs.
- Sternum stability, as indication for sternal bone union, will be clinically evaluated by an investigator, that was not involved in the surgery, and is blinded to the treatment arm. In case of suspected instability at end of study visit (6 months after surgery), a chest CT (non-contrast) will be performed.
- Surgical wound healing will be assessed by physical examination by an investigator, that was not involved in the surgery, and is blinded to the treatment arm. A modified Vancouver Scar Scale will be provided to the blinded investigators to prompt meticulous evaluation of surgical wound healing.
- Safety laboratory parameters: Routine hematology & chemistry.
- Urinalysis will be collected on screening and thereafter at investigator discretion.

8.7.1 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. Sternal Infection). Adverse events (AEs) will be collected from the time of enrolment, during follow-up visit until study termination at visit 10 (6 months after surgery).

The study Medical Monitor will review and categorize the cause of each Serious Adverse Event, including any Adverse Events suspected to be related to investigational medicinal product.

The study Medical Monitor will adjudicate if a safety reports meet the definition of SUSAR.

In case of dispute between investigator and the Medical Monitor, the more severe assessment will prevail.

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

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 48 of 98

Details on AE definition, assessment and reporting can be found in Section 11 “RECORDING & REPORTING OF ADVERSE EVENTS & REACTIONS”

8.8 LABORATORY EXAMINATIONS

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

If any local laboratory assessments are undertaken during the course of the trial and they result in a critical finding, (for example SAE or AE) the assessment data will be recorded in the patients’ eCRF (e.g, AE narrative).

8.8.1 CENTRAL LABORATORY

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

Complete clinical chemistry and hematology samples will be collected and shipped to the Central Laboratory according to laboratory manual. Clinical Chemistry and Haematology Parameters to be shipped to central laboratory

Clinical Chemistry Parameters
Albumin Alkaline Phosphatase Alanine Transaminase (ALT) Aspartate Aminotransferase (AST) Blood Urea Nitrogen (BUN) Calcium Chloride Creatinine C-Reactive Protein (CRP) Glucose Lactate Dehydrogenase (LDH) Phosphate Potassium Sodium Total Bilirubin Total Protein

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 49 of 98

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

8.8.2 LOCAL LABORATORY

Local reference range and accreditations will be collected for all local laboratories, and will be forwarded to be implemented in the eCRF.

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

Coagulation Parameters – to be done at Screening visit
Prothrombin Time (PT) Activated Partial Thromboplastin Time (aPTT) International Normalized Ratio (INR)
Urinalysis – to be done at Screening visit and thereafter, as per investigator discretion
pH Specific Gravity Protein Glucose Bilirubin Ketones Nitrites Leukocytes

Pregnancy test (β -HCG) by serum at screening only, performed in female of child-bearing potential only. All females of child bearing potential must agree to use of a highly effective method of contraception consistently and correctly for the duration of the study (see Section 6.3 CONTRACEPTIVE METHODS).

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.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 50 of 98

8.8.3 BACTERIOLOGICAL TESTING (SHOULD BE TAKEN BEFORE STARTING ANTIBIOTIC TREATMENT)

Bacteriological tests (from sternal bone and/or surrounding soft tissue and/or surgical wound discharge) will be performed in order to assess infection at the sternum surgical site.

The bacteriological specimen source and type should be indicated on source documents and eCRF.

Bacterial growth, identification and bacteria sensitivity to antibiotics (specifically to doxycycline and common practice antibiotics) will be assessed by a central laboratory.

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

8.9 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 (Wilson APR et al, 1986; Wilson APR et al, 1990).

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

8.10 PHARMACOKINETICS

8.10.1 THOROUGH PK ANALYSIS PROTOCOL

Thorough PK Study for assessing systemic exposure of D-PLEX will be performed.

8.10.1.1 Study Objectives

To assess PK systemic exposure of D-PLEX to be implemented during the course of this clinical trial.

8.10.1.2 Background

Systemic PK data will be obtained from patients treated with 3 vials of D-PLEX.

8.10.1.3 PK Study Underline Assumptions

Systemic Exposure of D-PLEX as measured by blood level of the drug substance - Doxycycline will be assessed.

C_{max} PK Parameter was selected for the analysis of inference due to:

- Most relevant PK parameter;

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 51 of 98

- Determined relatively in a short time following Extended Release medicinal drug topical administration (during T_{max} Time);
- Less affected by inter and intra-subjects variabilities;
- Less affected by patients co-morbidities and post cardiac sternotomy.

Other PK parameters will also be analysed.

Number of Vials to be used: The 3 vials group was selected, which represents the highest dose regimen and maximum Doxycycline Concentration (████ mg in total representing 3 D-PLEX vials of █████).

Although blood sampling will be taken at: baseline, 1, 2, 4, 6, 12, 24 hours, 48 hours (2 days), 72 hours (3 days), 120 hours (5 days), 336 hours (14 days) and 720 hours (1 month) post D-PLEX administration, the primary analysis of PK will be done from samples taken during the first 5 days under hospitalization (for better protocol adherence and compliance).

No. of patients: about █████ subjects treated with D-PLEX

Pharmacokinetic samples of █████nl blood will be collected for each PK time point, as detailed in the table below, prepared and shipped to central laboratory:

Visit / Day	Time after surgery	Comments
Surgery - Day 0 (Visit 2)	1. Pre-application of D-PLEX 2. After D-PLEX Application; 1 hour 2 hours 4 hours 6 hours 12 hours	Subject hospitalized
Day 1	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)	120 hours (Day 5)	
Day 14 (Visit 5)	Day 14	
Month 1 (Visit 6)	Month 1	

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 52 of 98

Laboratory requisition forms must be completed and samples must be clearly labelled with the subject number, protocol number, actual date / time sample was collected, site/center number.

Details for the preparation and shipment of samples, will be provided by the central laboratory in a separate instruction manual.

8.11 MEDICAL HISTORY AND PRIOR CONCOMITANT MEDICATIONS (VISIT 1, DAY-7 TO DAY 0)

Demographic data, cardiac and medical history of past, present illnesses and surgeries as well as medications currently being taken to address 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 should be excluded.

Subjects will be permitted to receive concomitant therapy as medically required, except for long term steroids use (defined as steroid use for over 3 consecutive months during a 6 month period), immunosuppressive agents and systemic doxycycline.

A record of all concomitant prescription medications will be maintained.

8.12 VITAL SIGNS (ALL VISITS)

Systolic/diastolic blood pressure, pulse, temperature will be recorded after 5 minutes of rest. Weight and height (taken at visit 1) will be taken from the subject, or the subject will be asked for this information at screening/admission.

All performed measurements will be recorded in CRF.

Any abnormal finding post study treatment, assessed by the investigator as clinically significant, should be recorded as AE in the relevant CRF section.

NOTE: Abnormal and/or CS will be assessed in accordance with subject condition.

8.13 LABORATORY EXAMINATIONS

Hematology and Chemistry samples will be collected at all visits , except of at visit 2)

Urinalysis will be collected at screening visit for all patients and at FU visits only at the discretion of investigator.

8.14 PHYSICAL EXAMINATION (SCREENING & VISITS 6, 8 & 10)

The investigator will perform a complete physical examination of the major body systems (Eyes, Ears, Nose and Throat; Cardiovascular; Respiratory; Gastrointestinal; Musculoskeletal/Connective Tissue; Neurological; Endocrine/Metabolic; Hematopoietic/Lymphatic; Dermatologic).

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 53 of 98

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.

8.15 ELECTROCARDIOGRAM (ECG) - (ONLY AT SCREENING)

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 ECG review is under the investigator's responsibility.

Any abnormal finding, assessed by the investigator as clinically significant, should be recorded under the medical history section in the relevant CRF form and source documents.

8.16 ALLERGY QUESTIONNAIRE (ONLY AT SCREENING)

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

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 54 of 98

8.17 SURGERY – INDEX PROCEDURE - VISIT 2

8.17.1 SOC OF ANTIBIOTIC PROPHYLAXIS IN CARDIAC SURGERIES

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

The SOC is based on:

The Society of Thoracic Surgeons practice guideline series: antibiotic prophylaxis in cardiac surgery, part II: antibiotic choice;

1. In patients for whom cefazolin is the appropriate prophylactic antibiotic for cardiac surgery, administration within 60 minutes of the skin incision is indicated. The pre-operative prophylactic dose of cefazolin for a patient of greater than 60 kg body weight is recommended to be 2g.
2. When surgical incision remains open in the operating room, to patients with normal renal function, a second dose should be administered every 3 to 4 hours. If it is apparent that cardiopulmonary bypass will be discontinued within 4 hours, it is appropriate to delay until perfusion is complete to maximize effective blood levels.
3. In patients for whom vancomycin is an appropriate antibiotic for cardiac surgery, a dose of 1 to 1.5 g or a weight adjusted dose of 15 mg/kg administered intravenously slowly over 1 hour, with completion with 1 hour of the skin incision, is recommended. A second dose of vancomycin of 7.5 mg/kg may be considered during cardiopulmonary bypass, although its usefulness is not well established.
4. For patients who receive an aminoglycoside (usually gentamycin, 4 mg/kg) in addition to vancomycin before cardiac surgery, the initial dose should be administered within 1 hour of the skin incision. Re-dosing an aminoglycoside during cardiopulmonary bypass is not indicated and may be harmful.

The standard of care of IV prophylactic antibiotics will be consistent for all sites that will participate in the clinical study.

The duration of the antibiotic prophylaxis may be up to the course of 24 hours.

8.18 SUBSEQUENT VISITS AND ASSESSMENTS (VISITS 3 – 10)

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 index procedure. These visits are usually performed after this kind of surgery.

In case a subject is in a medical hospitalization setting (e.g. rehabilitation center) 14 days post surgery, surgical wound assessment, adverse events and concomitant medication information for visit 5 (14 days post surgery) may be collected via a phone conversation with the medical treating staff.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 55 of 98

The additional follow up visits (visits 6-10) will take place at 1 month (30 days), 2 months, 3 months (90 days), 4 months and 6 months post index procedure and are required by the study protocol, i.e. in addition to the routine visits usually performed after this kind of surgery.

During these visits the following procedures will be performed:

- Vital signs,
- Assessment of sternal surgical wound – will be performed by an investigator that was not involved in the surgery, and is blinded to the treatment arm. A modified Vancouver Scar Scale will be provided to the blinded investigators to prompt meticulous evaluation of surgical wound healing. Clinical assessment of sternal stability.
- Laboratory examinations (Hematology & Chemistry).
- Adverse Events assessment and recording.

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

Bacteriology cultures will be taken in case of suspected sternal infection.

Full physical examination will be performed only at visits 6, 8 and 10.

Follow up assessments will be done over a total of 6 clinic visits until final assessment at 6 months $\pm$ 14 days.

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

9 SCHEDULE OF ASSESSMENTS

9.1 VISIT 1 (SCREENING, DAY -7)

Patient will be screened for study eligibility at the clinic within 7 days prior to surgery at Day 0 following ICF process and signature.

The following screening assessments will be performed:

- Informed consent process
- Medical History, Cardiac History, Demographics & Concomitant Medications prior to study
- Allergy Questionnaire
- Vital Signs, Weight and Height
- Physical Examination
- Chest X-Ray
- 12-lead ECG
- Blood Tests (hematology, chemistry, coagulation)
- Urinalysis
- Pregnancy Test (serum) for WOCBP
- Pain VAS Score assessment
- Eligibility Assessment

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 56 of 98

9.2 VISIT 2 (SURGERY, DAY 0)

The following will be performed on the surgery day:

- Vital Signs
- Randomization and allocation of treatment arm
- Pre-application Doxycycline PK sampling (for selected patients)
- D-PLEX Administration during sternum closure process for patients randomised to treatment arm
- Post-application Doxycycline PK sampling (for selected patients at 1h, 2h, 4h, 6h & 12h)
- Adverse Events & Concomitant Medication Review

9.3 FOLLOW UP VISITS 3-9

(DAY 1, DAY 5, DAY 14, 1 MONTH, 2 MONTHS, 3 MONTHS, 4 MONTHS)

- Vital Signs
- Blood Tests (hematology, chemistry)
- Post-application Doxycycline PK sampling (for selected patients – visits 3, 4, 5 & 6)
- Assessment of Infection per CDC definition (visits 3-8)
- ASEPSIS wound assessment (visits 3-8)
- Surgical Wound Healing Assessment (from visit 4)
- Clinical Assessment of Sternum Stability (from visit 4)
- Physical Examination (on visits 6 & 8)
- Pain VAS Score assessment
- Adverse Events & Concomitant Medications Review

9.4 VISIT 10 (END OF STUDY, 6 MONTHS)

- Vital Signs
- Blood Tests (hematology, chemistry)
- Surgical Wound Healing Assessment
- Clinical Assessment of Sternum Stability
- Physical Examination
- Pain VAS Score assessment
- Adverse Events & Concomitants Medication Review

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

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

9.5 TABLE OF STUDY ASSESSMENTS

Table 1: Study assessments

Procedures	Visit 1 Screening Day -7 to Day 0	Visit 2 surgery Day 0	Visit 3 Day 1†	Visit 4 Day 5†	Visit 5 ⁹ Day 14† (± 3 days)	Visit 6 1 month† (± 3 days)	Visit 7 2 month† (± 7 days)	Visit 8 3 month† (± 7 days)	Visit 9 ¹⁰ 4 month† (± 7 days)	Visit 10 Termination 6 month† (± 14 days)
Informed Consent	X									
Medical History & Allergy questioner completion	X									
Cardiac History and Status	X									X
Physical Exam	X					X		X		X
12-Lead ECG	X									
Urinalysis ²	X									
Chest X-Rays ^{7, 8}	X									
Vital Signs (blood pressure, HR, body temperature)	X	X	X	X	X	X	X	X	X	X
Weight & Height	X									
Pregnancy Test (serum)	X									
General Eligibility Criteria	X	Confirmation ⁵								
Randomization and Treatment Arm Allocation		X								

CONFIDENTIAL

Replace document:
CL-0016/05

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 58 of 98

Procedures	Visit 1 Screening Day -7 to Day 0	Visit 2 surgery Day 0	Visit 3 Day 1†	Visit 4 Day 5†	Visit 5 ⁹ Day 14† (± 3 days)	Visit 6 1 month† (± 3 days)	Visit 7 2 month† (± 7 days)	Visit 8 3 month† (± 7 days)	Visit 9 ¹⁰ 4 month† (± 7 days)	Visit 10 Termination 6 month† (± 14 days)
D-PLEX Administration		X								
Assessment of Infection per CDC definition ⁴			X	X	X	X	X	X		
ASEPSIS wound assessment			X	X	X	X	X	X		
Surgical wound healing assessment ⁶				X	X	X	X	X	X	X
Clinical assessment of sternum stability				X	X	X	X	X	X	X
Pain VAS Score assessment	X		X	X	X	X	X	X	X	X
Blood Tests (hematology, chemistry, coagulation ¹)	X		X	X	X	X	X	X	X	X
Doxycycline Pharmacokinetic Sampling ¹²		X	X	X	X	X				
Adverse Events Assessment and Recording	X ¹¹	X	X	X	X	X	X	X	X	X
Concomitant Medications Review and Recording	X	X	X	X	X	X	X	X	X	X
Study Termination										X ³

Notes:

- †. All visits' time points are calculated after surgery (which is considered as Day 0).
- 1. Coagulation is required only at screening, and thereafter at the discretion of the investigator during the study. Blood tests will not be collected on visit 2 (surgery day).
- 2. Urinalysis, will be done at screening visit for all patients, and thereafter at the discretion of the investigator during the study.
- 3. Termination procedure consists of: Termination Form completion and PI statement.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 59 of 98

4. In case of suspected sternal infection, Collection of bacteriology test – growth, identification and sensitivity to antibiotics (specifically to doxycycline and common practice antibiotics).
5. Investigator will confirm eligibility before randomization.
6. Surgical wound healing will be assessed by physical wound examination by an investigator blinded to study arm.
7. Chest X-Rays will be performed at screening. In case other chest imaging (i.e. CT/MRI) was performed, X-Ray may not be performed.
8. In case X-Ray (or CT/MRI) scan was performed within 3 months before surgery, and there was no significant change in subject's medical profile, this X-Ray (or CT/MRI) can be used for screening process.
9. In case a subject is in a medical hospitalization setting (e.g. rehabilitation center) 14 days post surgery, surgical wound assessment, adverse events and concomitant medication information for visit 5 (14 days post surgery) may be collected via a phone conversation with the medical treating staff.
10. In case subject was well, surgery wound healed and sternum stable, this visit may be done by phone and relevant information collected.
11. Collection of any SAE assessed as related to study participation (e.g., protocol-mandated procedures, invasive tests etc.)
12. Blood samples for Thorough PK will be collected from ■ patients treated with 3 vials of D-PLEX at the following time-points: baseline (pre D-PLEX application), 1, 2, 4, 6, 12, 24 hours, 48 hours (2 days; only if subject still hospitalized), 72 hours (3 days; only if subject still hospitalized), 120 hours (5 days), 336 hours (14 days) and 720 hours (1 month) post D-PLEX administration.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 60 of 98

9.6 DISCONTINUATION/WITHDRAWAL OF PARTICIPANTS & 'STOPPING RULES'

Every effort will be made to follow all patients and collect their data to the end of the study. 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, a 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 6 months 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 will be recorded (if given) 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 investigation 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 investigation 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.

10 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 (besides standard of care antibiotics as defined) will be administered as part of the requirements of this clinical study.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 61 of 98

10.1 STUDY TREATMENT D-PLEX

10.1.1 PRODUCT DESCRIPTION

D-PLEX is a new formulation of extended release of Doxycycline. The total percentage of doxycycline-hyclate in D-PLEX is [REDACTED] w/w, which is equivalent to [REDACTED] w/w doxycycline base. Doxycycline is being continuously released for approximately 3-4 weeks following administration.

D-PLEX is indicated for prevention of post cardiac surgery sternal infection.

10.1.2 PRODUCT COMPOSITION

D-PLEX contains the following substances:

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

10.1.3 PRODUCT USAGE AND DOSAGE

The product is to be administered on a single occasion prior to surgical wound closure following cardiac 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] g) may be administered. Therefore, the total amount of doxycycline free base for a single surgical procedure with D-PLEX can reach up to [REDACTED]

The individualized dose will follow the following scheme:

Sternum length (cm)	# of vials to apply
≤22	2
>22	3

D-PLEX will be applied directly within the surgical site immediately after hydration and mixing, in between and above the two halves of the sternum, during the process of sternum closure at the completion of the surgical procedure.

10.1.4 CONTRA_INDICATIONS

Please refer to the exclusion criteria (section 6.2).

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 62 of 98

10.1.5 USE IN SPECIFIC POPULATION

Please refer to the inclusion criteria in D-PLEX (section 6.1).

10.2 WARNING AND PRECAUTIONS

10.2.1 CLINICAL WARNINGS AND PRECAUTIONS

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

10.2.2 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 Certificate of Analysis and/or 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.

10.3 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 10-ml vial contains ■ 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:

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 63 of 98

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 “circulating” team member.

1. Remove D-PLEX product from the refrigerator.
2. Open the carton to expose the amber glass vial. **Caution: The outer surface of the vial is not sterile. Only the vial content is sterile.**
3. Make sure that the integrity of the box and vial was not damaged. **If it was damaged - do not use it.**
4. Take out the vial from the opened box.
5. Put a pair of sterile gloves.
6. Wipe the entire vial (including the cap) with alcohol swabs.
7. Vigourously shake the vial until a homogeneous powder is received.
8. Remove the vial cap by flipping the cap up and tearing it counter clockwise, according to the arrows stamped on the cap.
9. Open the vial slowly close to a sterile mixing surgical bowl, using standard aseptic techniques.
10. Pour the entire content of the vial (■) 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.**

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 64 of 98

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 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.
- Discard the empty vials of D-PLEX per local environmental standards.
- Document serial number of the used D-PLEX box and the number of used and discarded vials.

Step 3:

Should be done by the operating surgeon.

- **Administer** the whole quantity of the paste directly within the surgical site: in between and above the two halves of the sternum **immediately** after hydration and mixing.
- Packages (un-used vials and box) should be kept for inventory documentation purposes.
- Patients randomized to D-PLEX + SOC arm, which required sternal re-opening within 72 hours post primary operation due to other reasons than sternal wound infection, will be re-administered with D-PLEX after washing the previous applied D-PLEX.

10.3.1 OVERDOSAGE

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 ■■■ gr per patient). Overdose of D-PLEX will be recognized by the report of number of vials used as documented by the operation room (O.R.) team.

In case of an overdose or adverse drug reactions and per surgeon decision, the D-PLEX can be removed by re-exploring the surgical incision and washing out the D-PLEX. Such patients should not receive a second application of D-PLEX.

All overdoses should be recorded and notified to the sponsor.

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

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

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 65 of 98

10.3.2 PACKAGING

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

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

10.4 CONCOMITANT MEDICATION

10.4.1 MEDICATION(S)/TREATMENT(S) PERMITTED

- Concomitant Medications

All concomitant medications 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 (e.g. treatment of infection), according to investigator discretion.

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

10.4.2 MEDICATIONS PROHIBITED DURING THE TRIAL

- Any topical antibiotic is forbidden to be applied on the sternum and surgical incision/wound during or after surgery.
- Subjects will receive prophylactic IV antibiotics only per protocol. Additional antibiotics should be administered only in case of clinical need (e.g. treatment of infection), according to investigator discretion.
- Long term systemic steroids treatment (defined as steroid use for over 3 consecutive months).
- Immunosuppressive agents.
- Doxycycline IV/PO administration is not allowed in this protocol during the entire trial period.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 66 of 98

10.5 PREPARATION & LABELING OF INVESTIGATIONAL MEDICINAL PRODUCT (IMP)

The investigational medicinal product is manufactured aseptically at PolyPid, at a Grade A (ISO 5) clean room. The investigational medicinal product will be provided to the investigational sites by PolyPid Ltd., once Ethics Committee approval of the protocol and Informed Consent Forms have been received, Investigator Agreement is signed and all key documents required by GCP and local regulation have been received.

D-PLEX is provided sterile and primarily packaged in glass amber vial (10 ml vial), and each product vial contains 5g of D-PLEX.

The packaging will bear a statement that the drug is for clinical use in English and/or Local Language as required by GCP and local regulations.

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

10.6 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 subject dispensing records and 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 initials of the person dispensing the medication.

At study initiation, the monitor will evaluate the site's standard operating procedure for study drug disposal/destruction in order to ensure that it complies with PolyPid requirements. Drug may be returned or destroyed on an ongoing basis during the study as appropriate. At the end of the study, following final drug inventory reconciliation by the monitor, the study site will dispose of and/or destroy all unused study drug supplies, including empty containers, according to these procedures. If the site cannot meet PolyPid requirements for disposal, arrangements will be made between the site and PolyPid or its representative for destruction or return of unused study drug supplies.

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.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 67 of 98

11 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.

For the purpose of this study, all Sternal Infections will be collected and recorded on the "Sternal Infection" eCRF page.

11.1 DEFINITIONS

11.1.1 ADVERSE EVENT (AE)

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) 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 sequela of a suspected interaction of investigational medicinal product 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).

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 screening that do not worsen.
- Background disease/disorder, or expected progression, signs, or symptoms of the background disease/disorder, unless more severe than expected for the subject's condition.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 68 of 98

11.1.2 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).

11.1.3 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 as 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, diarrhoea, 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.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 69 of 98

11.1.4 UNEXPECTED ADVERSE EVENT

An adverse reaction the nature and/or severity of which is not consistent with the information about the medicinal product in question set out:

- in the case of a product with a marketing authorization, in the summary of product characteristics for that product,
- in the case of any other investigational medicinal product, in the investigator's brochure relating to the trial in question.

11.2 ASSESSMENT OF ADVERSE EVENT

Adverse event and serious adverse events will be reported in accordance with TIME PERIOD AND FREQUENCY FOR DETECTING AEs section 11.4

11.2.1 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.

11.2.2 CAUSALITY

Investigator's assessment of causality (relatedness of the event to the IMP or study procedure) is a clinical decision based on all available information at the time of the completion of the case report form. Investigator may change the causality assessment based on new information as additional tests, medical history etc. as available.

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

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 70 of 98

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.

11.2.3 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 available
Resolved	Subject has recovered and all AE/SAE's signs and symptoms resolved.
Resolved with Sequela	Subject has recovered, but subject retained pathological conditions resulting from the resolved AE/SAE. The sequela should not be reported as separate events, but should be described in the "General Narrative Comments" section of the AE/SAE reporting page.
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".
Lost to Follow-Up	Where an AE/SAE is ongoing at the time of subject being "Lost to Follow-Up"

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 71 of 98

11.2.4 EXPECTEDNESS

Category	Definition
Expected	An adverse event that is classed in nature as serious and which is consistent with the information about the IMP listed in the Investigator Brochure.
Unexpected	An adverse event that is classed in nature as serious and which is not consistent with the information about the IMP listed in the Investigator Brochure.

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

11.2.5 SERIOUSNESS

Seriousness as defined for an SAE in section 11.1.3.

11.3 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.

The Principal Investigator will 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 of first event or signs and symptoms, NOT the date the event became SAE.
3. Date of event becoming SAE
Report the start date the event became SAE. If signs and symptoms started prior to event turning into SAE, record them as an AE.
4. 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.
5. Seriousness criteria/s

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 72 of 98

6. Severity (mild, moderate, severe)
7. Relatedness to the surgical procedure
8. Relatedness to the IMP (Not Related, Unlikely Related, Possibly Related, Related)
9. Action taken to resolve the SAE (Treatment, Other Action)
10. Outcome (Ongoing, Resolved with Sequela, Resolved, Death, Lost to Follow-Up, Unknown)
11. Narrative of the event

All SAEs should be proactively followed up until resolution or permanent outcome of the event.

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

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

11.4 TIME PERIOD AND FREQUENCY FOR DETECTING AEs, SAEs AND EVENTS OF SPECIAL INTEREST (STERNAL INFECTION)

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 (e.g. Sternal Infection).

Adverse events (AEs) will be collected from the time of study treatment, during follow-up visit until study termination at visit 10 (6 months 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 a subject consents to participate in the study.

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

Type of Event	Initial Reports		Follow-up Information on a Previous Report	
	Time Frame from Knowledge	Documents	Time Frame from Knowledge	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

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 73 of 98

	Initial Reports		Follow-up Information on a Previous Report	
Type of Event	Time Frame from Knowledge	Documents	Time Frame from Knowledge	Documents
All Suspected and/or Confirmed Sternal Infection	24 hours	Sternal Infection data collecting tool	24 hours	Sternal Infection data collecting tool

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

Clinically significant abnormalities in the results of objective tests (e.g. laboratory variables, ECG, etc.) will also be recorded as adverse events. If the results are not expected as part of disease or IMP, these will also be recorded as an adverse event.

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

All adverse events will be recorded and will be reportable to sponsor up to and including 6 months, at Visit 10

11.4.1 PROCEDURES FOR RECORDING & REPORTING SERIOUS ADVERSE EVENTS

All serious adverse events will be recorded in the hospital notes and the eCRF, and the sponsor's AE log.

11.4.2 NOTIFICATION OF DEATHS

All deaths will be reported to the sponsor irrespective of whether the death is related to the baseline medical condition, surgery, the IMP, or an unrelated event. Deaths should be reported within 24 hours of knowledge to the sponsor.

11.4.3 ASSESSMENT OF ADVERSE EVENTS

The study Medical Monitor will review and categorize the cause of each Serious Adverse Event, including any Adverse Events suspected to be related to investigational medicinal product.

The Medical Monitor will adjudicate if a safety reports meets the definition of SUSAR.

A Data Monitoring Committee (DMC) will review all study safety data periodically until Last Patient Last Visit and provide its recommendation to the sponsor if study may continue based of patients' safety and any other ethical consideration as applicable.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 74 of 98

11.5 REPORTING OF SUSARs

The sponsor will assess expectedness of all SAE whose relationship to IMP is at least a reasonable possibility, i.e. the relationship cannot be rolled out.

The sponsor will notify the Investigators of all SUSARs. SUSARs that are fatal or life-threatening must be notified to the Investigators 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 to the ECs/IRBs and the Regulatory Authorities according to ICH-GCP and local regulations.

11.6 DEVELOPMENT OF SAFETY UPDATE REPORTS

The sponsor will provide all ECs/IRBs and the Regulatory Authorities with Safety Reports according to ICH-GCP and local regulations.

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

SAE reporting, 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 IRB/EC of SAEs (whether IMP related or not) as required by their Ethics Committee procedure.

Any SUSAR related to the IMP (D-PLEX) will need to be reported to the Sponsor irrespective of how long after IMP administration the reaction has occurred.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 75 of 98

12 NOTIFICATION OF SERIOUS BREACHES TO GCP 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.

The sponsor of a clinical trial shall notify the licensing 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 conduct phase.

13 DATA MANAGEMENT & QUALITY ASSURANCE

13.1 CONFIDENTIALITY

The Electronic Case Report Form (eCRF) will not bear the subject’s name or other personal identifiable data. The subject’s age and trial identification number, will be used for identification.

13.2 DATA COLLECTION TOOLS & SOURCE DOCUMENT IDENTIFICATION

Primary data collection based on source-documented hospital and/or clinic chart reviews will be performed clearly and accurately by site personnel trained on the clinical trial protocol and eCRF completion. The Sponsor or designee will 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.

13.3 DATA HANDLING & ANALYSIS

The procedures for data review and query management are described in the *Data Management Plan* and the *Edit Checks Specification Table*. Data will be reviewed throughout the study according to this document.

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 the corresponding local regulations in EU countries. 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.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 76 of 98

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 the eCRF could be considered as data source for this trial.

Subject identity should not be discernible from the data provided in 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 data source by the study monitor, and reviewed for consistency by Data Management using both automated logical checks and manual review.

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

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.

14 RECORD KEEPING & ARCHIVING

The Investigator shall arrange for the retention of study records for at least 15 years after completion or discontinuation of the study. Subject files and other source data shall be kept for the maximum period of time permitted by the hospital or institution.

The investigator should ensure that the following records are maintained:

- a) Subject files containing copies of completed case reports and supporting documentation and a copy of the signed, Informed Consent form.
- b) Investigator files containing copies of the documents required for the initiation of the study (signed Investigator's Agreement, Curricula Vitae for the principal and all sub-investigators, copy of the EC approval of the protocol and Informed Consent form), copies of correspondence received from and sent to CRO/Sponsor. In addition to these records required by regulations, CRO/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 (IMPAL) or an equivalent form approved by CRO/Sponsor, instructions for the use of the IMPAL and/or the Investigator's Brochure.

These documents should be retained for a longer period, if required by local or national regulations. **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.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 77 of 98

15 STATISTICAL CONSIDERATIONS

This section describes the statistical analysis as foreseen at the time of planning the study. Changes, additions, and further details about the analyses will be described in the Statistical Analysis Plan (SAP) signed and approved prior data base lock. After finalization of the SAP, any additional analyses or changes to analyses that may be required will be fully disclosed in the clinical study report (CSR).

15.1 STUDY DESIGN AND RANDOMIZATION PROCEDURE

This is a Phase III, prospective, multinational, randomized-controlled, two arm, single blind study. Due to the nature of the treatment, surgeons conducting the surgery cannot be blinded to treatment. Subjects will be randomized using in 1:1 ratio using dynamic randomization in attempt to balance the treatment groups for the following prognostic factors:

- Surgery duration of > 4 hours – yes/no
- Usage of double mammary artery bypass – yes/no
- BMI>40 – yes/no
- Co-existing of the co-morbidities (yes/no)
 - Yes : BMI≥30 AND Diabetes Mellitus AND (COPD or Current/Previous smoking history ≥30 pack year).
 - No : Otherwise

A subject's randomization assignment can only be obtained once consent and baseline information has been entered into the electronic data capture (EDS) system. In addition to randomization and blinding, study design will protect from bias by:

- a. Determination of the primary efficacy endpoint infection by an adjudication committee blinded to treatment arm;
- b. Determination of infection by blinded investigator;
- c. All site study staff except the study staff attending the surgery, will be blinded to treatment arm.

15.2 SAMPLE SIZE CONSIDERATION

15.2.1 POWER CALCULATION

Determination of sample size is based on demonstrating superiority of D-PLEX to SOC on the primary endpoint of infection rate at up to 90 days post-surgery. It is assumed that population infection rates are [REDACTED] and [REDACTED] in SOC and D-PLEX respectively reflecting [REDACTED] reduction in the risk. In addition, a [REDACTED] death rate is estimated for both treatments and will be considered as failure (infection), thus yielding [REDACTED] and [REDACTED] infection rates for SOC and D-PLEX arms respectively.

The following assumptions were used for the sample size:

- Randomization will allocate patients to D-PLEX and SOC in a 1:1 ratio
- Infection rates of D-PLEX and SOC are [REDACTED] and [REDACTED] respectively, yielding a relative risk of [REDACTED] reflecting [REDACTED] effect reduction in the risk.
- One Interim Analysis (IA) is expected approximately when [REDACTED] of the subjects complete 3 months (90 days) Follow-up. This IA will be designated to stop the study for futility or proven efficacy.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 78 of 98

- Experiment-wise two sided [REDACTED]
- [REDACTED] lost to follow-up rate

Under the above assumption, a sample size of [REDACTED] evaluable patients in total ([REDACTED] per group) will provide [REDACTED] power to demonstrate superiority of D-PLEX over SOC. The sample size was determined using the Fisher's exact test.

15.2.2 BLINDED SAMPLE SIZE REASSESSMENT

15.2.2.1 Timing:

The blinded non-comparative sample size reassessment will be performed after a total of N = [REDACTED] subjects (constitute approximately [REDACTED] of the minimal total sample size), have been assessed for primary efficacy (infection within 90 days) analysis.

15.2.2.2 Maximal Sample Size:

The maximal sample size allowed to upsize the study will not exceed [REDACTED] subjects. This sample size was based on clinical meaningful effect prorated in acceptable infection rate.

15.2.2.3 Procedure for blinded non-comparative sample size adaptation:

Step 1: Estimate the SOC Infection Rate (IR) + Death Rate (DR) in a fully blinded manner using the assumption of the treatment effect from the design stage (i.e. [REDACTED] risk reduction). That is, given the pooled observed Infection Rate + Death Rate with the assumed [REDACTED] treatment effect, the SOC IR + DR will be extracted.

Step 2: Calculate the pooled observed lost to follow.

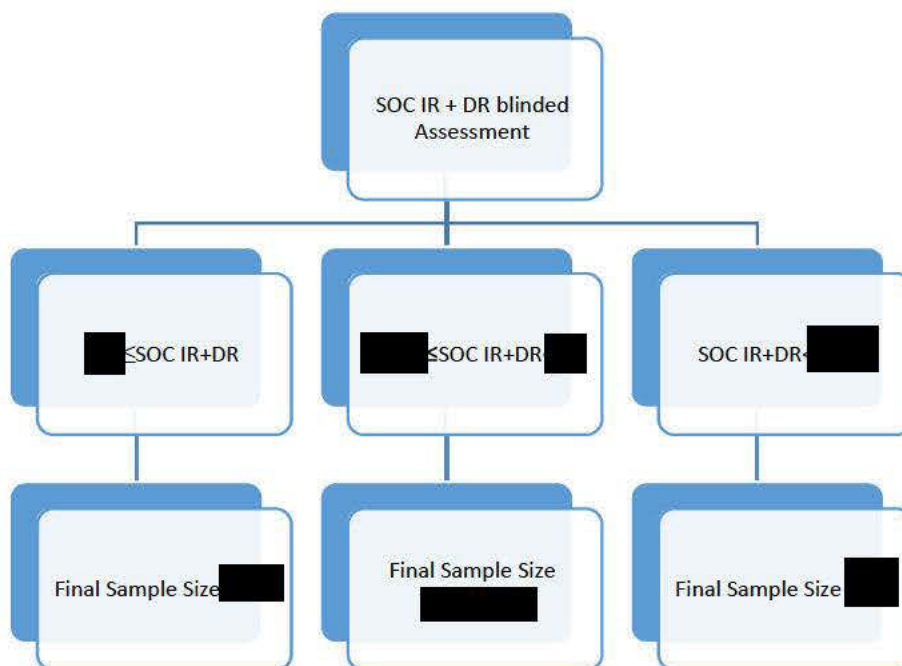
Step 3: Using the above updated SOC IR+DR as well as the observed lost to follow-up, a new power calculation will be performed while keeping all other assumptions, which were used for the sample size calculation, similar as in the design stage (i.e. significance level, assumed treatment effect, method of analysis etc..).

Step 4: Decision Rules:

As shown in the figure below, the following rules will guide the final sample size determination:

- If the re-estimated SOC IR+DR $\geq$ [REDACTED] and the recalculated power $>$ [REDACTED], then no change in the sample size will be made;
- If the re-estimated SOC IR+DR is between [REDACTED] and the recalculated power $<$ [REDACTED], then the sample size will be increased up to [REDACTED] subjects to reach [REDACTED] power. The rate of [REDACTED] corresponds to [REDACTED] subjects for [REDACTED] power if all other assumptions mentioned for the sample size calculations are the same;
- If the re-estimated SOC IR + DR is $<$ [REDACTED], then the maximal sample size of [REDACTED] subjects will be used;
- The sponsor reserves the right not to change the original sample size of [REDACTED] subjects if the SOC infection rate is slightly less (up to [REDACTED]) than the original [REDACTED]

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------



15.3 INTERIM ANALYSIS

A formal interim analysis to evaluate efficacy and futility will be conducted after a total of $N =$ [redacted] subjects (constitute approximately [redacted] of the minimal total sample size), have been assessed for primary efficacy (infection within 90 days) analysis. In case the sample size will be increased following blinded non-comparative sample size adaptation, the number of subjects included in the interim analysis will be increased accordingly to constitute approximately [redacted] of the updated total sample size.

The futility stopping rule will be formulated using Conditional Power (CP) such that: If $CP <$ [redacted] then the study could be declared futile and be terminated. This stopping rule was chosen to spend [redacted] of the risk (Type II error) at the interim out of the overall [redacted] in the study. The CP will be calculated assuming the design assumptions on the unobserved data.

The efficacy stopping rule will be formulated by one sided p-value using the published Lan Demetz O'Brien Fleming error spending function such that if the one sided p-value $<$ [redacted] (or two sided $<$ [redacted]), then the study will be stopped for proven efficacy. The level of significance at the end of the study will be [redacted]. The p-value will be calculated using Fisher's Exact test.

All of the above calculations were performed using EAST software v6.4.

The interim analysis will be conducted by an Independent Biostatistician (external to sponsor). Based on this analysis, the DMC will recommend for futility or proven efficacy if the above rule is met together with other efficacy and safety data to support it.

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 80 of 98

15.4 ANALYSIS SETS

15.4.1 INTENTION TO TREAT (ITT)

Intent to treat (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 received.

15.4.2 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

15.4.3 MODIFIED INTENTION TO TREAT (mITT)

The Modified ITT 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 at study entry.

15.4.4 PER PROTOCOL ANALYSIS SET

The per protocol (PP) analysis set is a subset of mITT analysis set with no major protocol violations, with valid 90-day observations on the primary endpoint. "Major" violation will be defined as one having the potential to affect study outcome and will be specified in the SAP.

15.5 PATIENT DISPOSITION

Data from patients screened, patients screened but not randomized, patients in the ITT, safety, mITT and PP analysis set, patients who complete the study, and patients who withdraw from the study will be summarized using descriptive statistics. Data from patients who withdraw from the study will also be summarized by reason for withdrawal using descriptive statistics.

15.6 DEMOGRAPHIC AND BASELINE CHARACTERISTICS

Patient demographic and baseline characteristics will be examined to assess the comparability of the treatment groups and will be summarized using descriptive statistics. Observed differences between the groups, should there be any, will be interpreted for their clinical significance and their potential use as covariates in the analysis of efficacy endpoints.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 81 of 98

15.7 END POINTS

15.7.1 PRIMARY EFFICACY ENDPOINT

The primary efficacy endpoint is the Infection rate as measured by the proportion of subjects with a sternal wound infection event within 90 days (3 months) post sternotomy for cardiac surgery.

Sternal infection is composed of Deep Sternal Wound Infection (DSWI) and Superficial Sternal Wound Infection (SSWI).

Mortality from any reason within 90 days (3 months) post sternotomy or missing data, will be analyzed as treatment failure (infection=1).

For each subject, a dichotomously scored variable will be created:n:

- Infection = 1 (yes) = presence of sternal surgical site infection up to 90 days post-surgery or all cause mortality or missing data;
- Infection = 0 (No) = Otherwise.

15.7.2 KEY SECONDARY EFFICACY ENDPOINTS

Following are the study's secondary endpoints. The endpoints are listed in the order in which the analyses will be performed according to the gate keeping approach to control for multiplicity.

1. Average number of Hospitalization days post sternotomy due to sternal surgical site infection.
2. Average ASEPIS assessment score during 90 days.
3. Number of surgical re-interventions due to Sternal Surgical Site infection.

15.7.3 'OTHER SECONDARY' ENDPOINTS (RECOMMENDED BY REGULATORS)

1. Incidence of SSWI rates during 90 days post sternotomy.
2. Incidence of DSWI during 90 days post sternotomy.
3. Mortality rate associated with sternal wound infection within 90 days (3 months) post sternotomy.
4. Proportions of patients with susceptibility to Doxycycline of any organisms recovered from a Sternal Surgical Site Infection Site.

15.7.4 ADDITIONAL SECONDARY EFFICACY ENDPOINTS

1. Overall number of hospitalization days, for any reason.
2. Number of readmissions due to Sternal Surgical Site infection.
3. Average number of Antibiotic Treatment (overall IV and other administration modes, e.g. oral) days due to Sternal Surgical Site infection (SSWI & DSWI).
4. Average number of days with Antibiotic Treatment (IV) days due to Sternal Surgical Site infection (SSWI and DSWI).
5. Time to Sternal Surgical Site Infection (Post Operating Day) post sternotomy.
6. Average number of analgesic treatment days.
7. Pain VAS Score assessment.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 82 of 98

15.7.5 SAFETY ENDPOINTS

The following safety parameters will be evaluated in this trial:

- Adverse events, physical examinations & vital signs.
- Sternum stability, as indication for sternal bone union, will be clinically evaluated by an investigator that was not involved in the surgery, and is blinded to the treatment arm. In case of suspected instability at end of study visit (6 months after surgery), and pending patient's clinical status allows to perform imaging, a chest CT will be performed.
- Incisional wound healing will be assessed by physical examination by an investigator blinded to study arm. 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: Routine hematology, chemistry. Urinalysis will be collected on screening and thereafter at investigator discretion.

15.8 CONTROLLING OF TYPE I ERROR

The overall significance level for this study is [REDACTED] using two-tailed tests and/or two-sided confidence intervals with [REDACTED] confidence level. Due to interim analysis for efficacy and using the published Lan Demetz O'Brien Fleming error spending function the level of significance at the end of the study for the primary analysis will be [REDACTED]

In order to protect the study from type-I error inflation, the secondary endpoints will be interpreted inferentially only if a statistically significant treatment effect is detected in the primary analysis. Type-I error will be further controlled by employing the Hierarchical Approach, (i.e. each secondary endpoint will be analyzed only in case the preceding endpoint will have a p-value less or equal to 0.05 for D-PLEX over SOC comparison) according to the order specified in Section 15.7.2.

15.9 STATISTICAL ANALYSES

15.9.1 OVERVIEW

Safety analyses will be conducted on the safety analysis set. In addition, for AE's only the ITT analysis set will also be used. All efficacy testing will be done using the ITT analysis set. mITT and Per Protocol (PP) analysis sets will be used as sensitivity for the primary analysis

For continuous variables, descriptive statistics (number, mean, SD, standard error, median, minimum, and maximum) will be provided. For categorical variables, patient counts and percentages will be provided. Categories for missing data will be presented if necessary. Tables will be presented by visit, study arm and overall. Data listing will be provided by subject.

All statistical analyses will be performed and data appendices will be created using the SAS® v9.4 or later .

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 83 of 98

15.9.2 PRIMARY EFFICACY ANALYSIS

Primary analysis will be done on the ITT analysis set. The null hypothesis and alternative are as follows:

H0: Infection Rate_{D-PLEX} = Infection Rate_{SOC}

H1: Infection Rate_{D-PLEX} ≠ Infection Rate_{SOC}

The primary end-point of sternal surgical site infection up to 90 days post-surgery will be analysed using Fisher's exact test with two-sided Alpha- (due to interim analysis for efficacy). Patient with missing data or mortality from any cause for the primary endpoint will be treated as failure. That is, Infection = 1 (yes) - presence of sternal surgical site infection.

The heterogeneity of treatment effects across sites will be explored by separately testing the treatment by site interaction using proc logistic with STRATA and EXACT option. This test will be performed in an exploratory fashion, and at a nominal significance level of .

15.9.3 SENSITIVITY FOR PRIMARY EFFICACY ANALYSIS

The following sensitivity analyses will be performed to evaluate the robustness of the primary analyses

- The primary analysis will be repeated using the mITT and PP analysis set
- The primary analysis on the ITT will be repeated excluding mortality of any cause as failure
- The primary analysis will be repeated stratifying for gender using Cochran–Mantel–Haenszel test (CMH)
- **Handling Missing Data** - Subjects missing primary data and who did not have infection on their observed measurements will be imputed success (infection = 0) on the primary endpoint. This is equivalent to the last observation carried forward (LOCF) method, since only subjects with no infection observed will be imputed; those lost to follow-up who had infection at any point before dropping out of the trial will be coded failures. The following additional patterns of missingness will be analysed for the primary endpoint:
 - Best Case: Where missing values will be imputed Success (no infection) in the D-PLEX arm and Failure in Control
 - Worst case: where missing values will be imputed as failure (infection) in D-PLEX and success in Control
 - Tipping point, where missing values will be progressively imputed as Failure in the D-PLEX arm and Success in the Control

Imputations will be conducted until result that had been significant in favour of D-PLEX will no longer be significant. The tipping point is defined at the point where the significant result becomes non-significant.

The effects of noncompliance, dropouts, and possible covariates such as gender, and their interactions with treatment, will be assessed to determine the impact on the general applicability of results from this study.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 84 of 98

15.9.4 KEY SECONDARY ANALYSES

The key secondary analyses, involving the key secondary endpoints will be tested according to the hierarchical order specified in Section 15.7.2.

1. The analysis of number of Hospitalization days post sternotomy due to sternal surgical site infection will be based on the outcome of a contrast (D-PLEX+SOC vs. SOC) derived from Poisson Regression (SAS® PROC GENMOD with DIST=POISSON). In addition to treatment group, the model will include the four stratification factors used in the dynamic randomization as covariates:
 - Surgery duration of > 4 hours – yes/no
 - Usage of double mammary artery bypass – yes/no
 - BMI>40 – yes/no
 - Co-existing of the co-morbidities (yes/no)
 - Yes : BMI≥30 AND Diabetes Mellitus AND (COPD or Current/Previous smoking history ≥30 pack year).
 - No : Otherwise
2. The analysis of average ASEPSIS assessment score during 90 days will be based on the outcome of a contrast (D-PLEX+SOC vs. SOC) derived from analysis of variance (ANOVA SAS® MIXED procedure). In addition to treatment group, the model will include the four stratification factors used for the dynamic randomization as covariates (similar to the description in the number of hospitalization analyses).
3. The analysis of the number of surgical re-interventions due to Sternal Surgical Site infection will be based on the outcome of a contrast (D-PLEX+SOC vs. SOC) derived from Poisson Regression (SAS® PROC GENMOD with DIST=POISSON). In addition to treatment group, the model will include the four stratification factors used for the dynamic randomization as covariates (similar to the description in the number of hospitalization analyses).

15.9.5 'OTHER SECONDARY' ANALYSES (RECOMMENDED BY REGULATORS)

The following other secondary endpoints were recommended by regulators and will be analyzed in a descriptive manner. No inferential statistics will be conducted and p-values may be provided nominally for trends detection.

1. The analysis of Incidence of SSWI rates during 90 days post sternotomy will be analyzed using Fisher's Exact test similarly to the primary end-point.
2. The analysis of Incidence of DSWI during 90 days post sternotomy will be analyzed using Fisher's Exact test similarly to the primary end-point.
3. The analysis of Mortality rate associated with Sternal Wound Infection (SWI) within 90 days (3 months) post sternotomy will be analyzed using Fisher's Exact test similarly to the primary end-point.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 85 of 98

4. The analysis of proportions of patients with susceptibility to Doxycycline of any organisms recovered from a Sternal Surgical Site Infection Site will be based on the outcome of a contrast (D-PLEX+SOC vs. SOC) derived from logistic Regression (SAS® PROC GENMOD with DIST=LOGIT).
5. Average number of Antibiotic Treatment (overall IV and other administration modes, e.g. PO) days due to Sternal Surgical Site infection will be analyzed using Analysis of Covariance (ANCOVA).

Further details on each analysis will be provided in a Statistical Analysis Plan signed and approved prior to data base lock.

15.9.6 ADDITIONAL EFFICACY ANALYSES

The following are additional efficacy exploratory endpoints that will be analyzed in exploratory manner. No inferential statistics will be conducted and p-values may be provided nominally for trends detection.

- The analysis of the overall number of hospitalization days due to any reason will be based on the outcome of a contrast (D-PLEX+SOC vs. SOC) derived from Poisson Regression (SAS® PROC GENMOD with DIST=POISSON).
- The analysis of the number of readmissions due to Sternal Surgical Site infection will be based on the outcome of a contrast (D-PLEX+SOC vs. SOC) derived from Poisson Regression (SAS® PROC GENMOD with DIST=POISSON).
- The analysis of the number of days with Antibiotic Treatment (overall IV and other administration modes, e.g. oral) due to Sternal Surgical Site infection; SSWI and DSWI will be based on the outcome of a contrast (D-PLEX+SOC vs. SOC) derived from analysis of covariance (ANCOVA SAS® MIXED procedure).
- The analysis of the Number of days with Antibiotic Treatment (IV) due to Sternal Surgical Site infection (SSWI and DSWI) will be based on the outcome of a contrast (D-PLEX+SOC vs. SOC) derived from analysis of covariance (ANCOVA SAS® MIXED procedure).
- The analysis of Time to Sternal Surgical Site Infection (Post Operating Day) post sternotomy will be analyzed using log rank test. Kaplan Meier figure will be created.
- The analysis of the number of days with analgesic treatment will be based on the outcome of a contrast (D-PLEX+SOC vs. SOC) derived from analysis of covariance (ANCOVA SAS® MIXED procedure).
- The analysis of Pain VAS Score assessment at month 3 –will be based on the outcome of a contrast (D-PLEX+SOC vs. SOC) derived from analysis of covariance (ANCOVA SAS® MIXED procedure).

Further details on each analysis will be provided in a Statistical Analysis Plan signed and approved prior to data base lock.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 86 of 98

15.9.7 ANALYSES OF EFFECT HOMOGENEITY

Poolability analyses will be tested test homogeneity of the effect across regions and sites within regions as well as across IMP manufacturing sites. The primary endpoint will be analysed using logistic regression with the following each covariate of interest together with the interaction with treatment.

Region coded dichotomously as US/OUS (Out of US)

- Site within US
- Site within OUS
- IMP manufacturing site (two levels)

The effect of interest in this model is the Covariate X Treatment-Arm interaction, indicating whether the effect of Treatment is dependent on levels of the covariate.

15.9.8 SAFETY

Safety analyses will be conducted on the safety analysis set and will be descriptive in nature. In addition, for AE's only the ITT analysis set will also be used. All adverse events (AEs), including all serious adverse events (SAE's) and all (SUSAR's) will be summarized and tabulated by preferred term within a body system, both overall and by severity, by treatment arm, by relation to treatment, using the MedDra dictionary. For tabulation by severity only a subject's most severe event within the category will be counted. Special attention will be paid to the safety endpoints enumerated in Section 5.2.

16 NAME OF COMMITTEES INVOLVED IN TRIAL

16.1 DATA MONITORING COMMITTEE (DMC)

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

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

There will be no summary of the study by the two treatment arms separately, with the exception of a DMC assessment in the course of an interim/futility analysis.

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

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

16.2 END-POINTS ADJUDICATION COMMITTEE (EPAC)

An independent of the sponsor/investigator involved in the study, and blinded to treatment arm "End-Point Adjudication Committee" will be established.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 87 of 98

All information collected regarding efficacy (sternal infection) will be reviewed by the “End-Point Adjudication Committee”. In addition, the committee will review all death cases to verify if they may be related to sternal infection, IMP or other cause.

In case of dispute between investigator assessment of infection and the committee’s assessment, End-Point Adjudication Committee’s assessment on primary end point evaluation will overrule the Investigator assessment.

17 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 investigation.

The investigator will permit trial-related monitoring, audits, EC 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.

18 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 and the Sponsor before subject enrolment. Changes to the protocol must be approved in writing by the Sponsor and the EC (as applicable) before the change is implemented.

Prior to subject enrolment, a signed copy of the EC 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 and forwarding copies of the approval letters to the Sponsor. The original letters are to be kept in the investigational center’s Regulatory Binder designated for this study.

The investigator will notify the Sponsor immediately, no latter than within five (5) working days of withdrawal of EC approval.

19 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) agrees to allow the monitor direct access to all relevant documents and to allocate their time and the time to their staff to monitor to discuss findings and any issues.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 88 of 98

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.

20 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.

21 INSURANCE

The Sponsor will provide insurance or indemnify (legal and financial coverage), the Investigatory/Institution against claims arising directly from the study drug or a properly performed procedure required by the study plan, insurance covering the reasonable cost of treatment of the subjects in the event of clinical-investigation-related injuries, in accordance with national laws and 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.

22 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 these 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.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 89 of 98

23 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 investigation shall not begin until the required approval or favorable opinion from the EC has been obtained and, if applicable, any local or national regulatory authority approvals or notifications have been obtained.

The Sponsor has obtained clinical investigation insurance. This insurance covers the possibility of physical damage or injuries that are a direct results of the treatments performed according to protocol.

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 90 of 98

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CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 91 of 98

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CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 92 of 98

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CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

25 APPENDICES

25.1 ASEPSIS SCORE ASSESSMENT

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

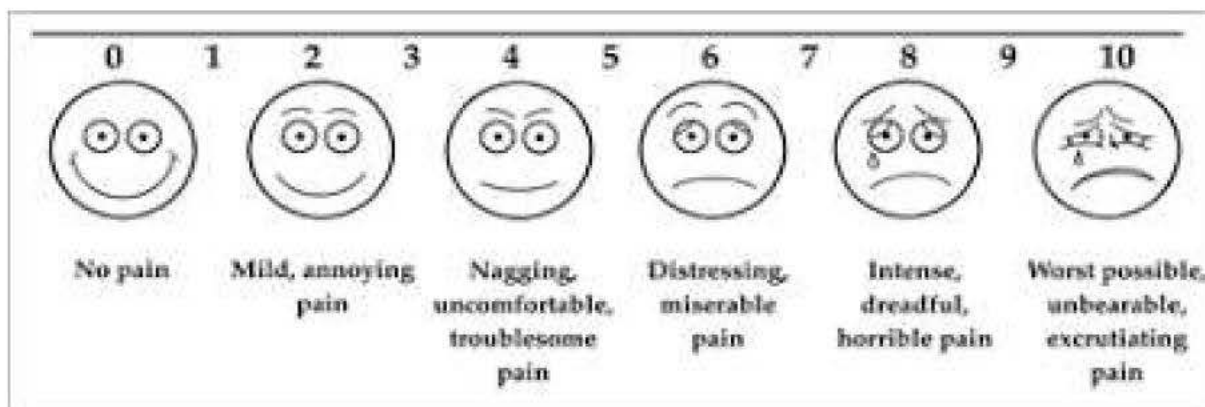
Table 1. ASEPSIS wound score outline^[1]

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

25.2 PAIN VAS SCORE

The number that the responded indicates on the scale to rate their pain intensity is recorded. Scores range from 0-10.

Higher scores indicate greater pain intensity.



	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 94 of 98

25.3 MODIFIED VANCOUVER SCAR SCALE

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

Lower scores indicate better wound healing.

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

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 95 of 98

25.4 DOCUMENT CHANGE HISTORY

Revision	Changes	Reason for changes
Version 05	Section 9.5 - Table of study assessments – “Notes” was updated to detail Thorough and ADD on PK sampling time-points (Note #12)	Clarifying PK sampling requirements
	Summary, Study design The SOC antibiotic prophylaxis to be used in the study, was detailed	Adjustment of the protocol summary to the protocol content
	Section 6.3 Section was added to provide details on contraceptive methods	CTA (VHP)comment
	Update of contact table: Replacement of Data Management/Data Analysis, Added Central CT reading, additional CRO and additional locations of ICON laboratories.	New information
	Section 11.2.3 Update the definition of "Lost to Follow-Up" category	Deletion of irrelevant information.
	Sections 8.6.3 and 16.2 End-Point Adjudication Committee’s decisions will not be forwarded to investigator and to PolyPid.	To ensure unbiased assessment of end-point by the investigator and the adjudication committee

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 96 of 98

	Section 8.10.2.1 Addition of “The time-point for the PK sample collection will be allocated by the randomization system (IWRS)”.	Clarification of the time-point for Pop PK sample collection
	Sections 21 and 23 Rewording of insurance information.	Clarification
	Section 8.13 Removal of duplicate text.	Redundancy
	Through the protocol	Typo corrections
Version 04	Sponsor representative details changed	Company staff changes
	Clarification that D-PLEX may be re-applied in case of sternal re-opening	Clarification
	Section 10.1.3 – Correction of “abdominal surgical procedures” to “cardiac surgical procedures”	Correction of typographical error
	Section 3.4.1 – Removal of risks of D-PLEX in sternal surgeries: “Potential contamination of microorganisms and pyrogens by the aseptic product.” “Administration outside of the target organ- sternal surgery site.” “Inadequate anti-bacterial activity of the product”	Risks are not related to D-PLEX

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 97 of 99

	“Re-use of D-PLEX between different patients” “D-PLEX removed from the target organ following re-intervention”	
Version 03	Typographical error (on summary, 6.1, 6.2, 15.2.2.3 & 15.3) corrections	Corrections
Version 02	Only unblinded site staff can provide information on the treatment arm in case of emergency	In order to protect the integrity and validity of the study
	Subjects’ age will be collected instead of date of birth	GDPR compliance
	Addition of Vancouver Scar Scale for evaluation of surgical wound healing	To uniform wound healing assessment, further to FDA advice
	Added details of exclusion criteria # 12 “Subjects scheduled for mechanical assist device”	Clarification
	Following appendices were added: ASEPSIS scale , Vancouver Scar Scale & Pain VAS Score (including clarification this is Pain VAS).	Update
	Stratification for gender was switched to balanced randomization for other parameters	In order to ensure balanced treatment arms further to EMA advice

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------

	D-PLEX 302 Clinical Trial Protocol		
	Doc. No. CL-0016	Version: 06	Page: 98 of 98

	Statistical section was updated to correlate with balanced randomization Re-phrasing and re-wording of statistical sections	Update and clarify the statistical process
	Re-phrasing and re-wording of “study blinding section”	To emphasize the blinding requirements
	Update of time period and frequency for detecting AEs, SAEs and events of special interest (sternal infection) section	AE casualty report within 24 hours is not required
	Wording and page layout corrections	Typographical errors

CONFIDENTIAL	Replace document: CL-0016/05
---------------------	---------------------------------