STATISTICAL ANALYSIS PLAN

Trial ID: PB1046-PT-CL-0003-P1

PHASE 2A, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, MULTIPLE-DOSE, 2-PART STUDY TO ASSESS THE SAFETY, TOLERABILITY AND PHARMACOKINETIC RESPONSE AND EXPLORE THE PHARMACODYNAMIC RESPONSE FOLLOWING 4 WEEKS OF ONCE WEEKLY SUBCUTANEOUS INJECTIONS OF PB1046 IN ADULT SUBJECTS WITH STABLE HEART FAILUREWITH REDUCED EJECTION FRACTION (HFREF) (PART 1) AND IN SUBJECTS WITH CARDIAC DYSFUNCTION SECONDARY TO DUCHENNE OR BECKER MUSCULAR DYSTROPHY (PART 2)

This plan cover part 1 only

Approval:	Lei Shi			12-13-2017
	IMD Statistician	Signature		Date
Approval:		John Lee		12-13-2017
	Medical Monitor	Signature		Date
Approval:	Sheng Xu			12-13-17
	Phasebio Statistician	Signature		Date

Table of Contents

ABBREVIATIONS

Abbreviations	Definitions
ADA	Anti-drug Antibodies
AE	Adverse Event
BMI	Body mass index
BSA	Body surface area
BP	Blood Pressure
Cmax	Maximum Serum Concentration
CFB	Change from baseline
DLT	Dose Limiting Toxicity
DBP	Diastolic blood pressure
ECG	Electrocardiogram
ECHO	Echocardiography
eCRF	electronic Case Report Form
eGFR	Estimated glomerular filtration rate
ITT	Intent-to-Treat
OSBP	Office-seated Blood Pressure
PB1046	Vasomera
PD	Pharmacodynamic
PK	Pharmacokinetic
PP	Per-Protocol
PT	Preferred Term
QTcB	QT interval corrected for heart rate
QTcF	QT corrected by Fredericia's formula
SAE	Serious adverse event
SBP	Systolic blood pressure
SC	Subcutaneous
SD	Standard Deviation
SMBP	Self-monitored blood pressure
SOC	System Organ Class
SRC	Study Review Committee

ABBREVIATIONS

Abbreviations	Definitions
ADA	Anti-drug Antibodies
TEAE	Treatment emergent adverse event

1 OVERVIEW

1.1 INTRODUCTION

This documentation describes the data analyses for PhaseBio PB1046-PT-CL-0003-P1 clinical trial.

1.2 OBJECTIVES

1.2.1 Primary Objective

To evaluate the safety and tolerability of escalating doses of PB1046 administered once weekly for 4 weeks in adult subjects with stable NYHA Class II/III heart failure with a reduced ejection fraction (HFrEF).

1.2.2 Secondary Objectives

- Characterize the pharmacokinetic profile of a subcutaneous dose of PB1046 administered once weekly for 4 weeks;
- Evaluate the immunogenicity profile of a subcutaneous dose of PB1046 administered once weekly for 4 weeks.

1.2.3 Exploratory Objectives

- Characterize the effect of escalating doses of PB1046 on cardiac function as measured by 2-D echocardiography with Doppler;
- Characterize the effect of PB1046 on cardiac, anti-inflammatory and anti-fibrotic biomarkers.

2 INVESTIGATIONAL PLAN

2.1 STUDY DESIGN AND RANDOMIZATION

2.1.1 Study Design

This study is a randomized, double-blind, placebo-controlled study that will assess the safety, tolerability, pharmacokinetics and explore the pharmacodynamics of PB1046 following 4 weeks of once weekly subcutaneous injections.

This study will be conducted in two parts:

- Part 1 will be a sequential multiple-dose escalation study that will enroll (randomize and dose) approximately 28 (3A:1P in Cohort 1 and 6A:2P in subsequent cohorts) subjects total of 4 cohorts.

- Part 2 of the study will be further described and conducted under a separate Part 2 protocol (PB1046-PT-CL-0003-P2).

2.1.2 Inclusion Criteria

Subjects will be eligible for inclusion in the study if they meet all of the following criteria:

1. Willing and able to sign a written informed consent and follow all study-related procedures;

2. Male or female subject ≥ 18 years of age;

3. Male subjects and female subjects of reproductive or childbearing potential must practice effective contraception during the study and be willing and able to continue contraception for 30 days after their last dose of study drug. Female subjects of non-childbearing potential are defined as being surgically sterile by bilateral tubal ligation, bilateral oophorectomy, or hysterectomy. A female subject 45 to 60 years of age, inclusive who is post-menopausal for at least 1 year and has a follicle-stimulating hormone level confirmation indicating post-menopausal status will be considered of non-childbearing potential. Female subjects > 60 years of age are considered post-menopausal and of non-childbearing potential;

4. Body mass index ≥ 18 kg/m2 and ≤ 45 kg/m2;

5. Receipt of stable (no change in dose (except diuretics) or addition or removal of a therapy) pharmacological therapy(ies) for heart failure for a minimum of 1 month (30 days) prior to screening and between screening and randomization and are in stable clinical condition;

6. NYHA Class II or III heart failure with reduced ejection fraction diagnosis confirmed by medical history at least (≥) 6 months (180 days) prior to screening and/or ischemic or non-ischemic cardiomyopathy with reduced ejection fraction confirmed by medical history at least (≥) 6 months (180 days) prior to screening and historical echocardiography of ejection fraction ≤ 40% prior to screening;

7. Stable HF defined as no hospitalizations for cardiac related issues within the previous 3 months (90 days) prior to the screening visit or between screening and randomization;

8. Screening hemoglobin ≥ 9.0 g/dL secondary to the volume of blood to be collected during the study period.

9. Willing and able to return to the study unit for specified study visits, and be able to self-monitor blood pressure while at home.

10. Live and work in an area with reliable cellular services (e.g., Sprint®) for real time transmission of telemetry data to the core laboratory.

2.1.3 Exclusion Criteria

Subjects will be excluded from the study if they meet any of the following criteria:

1. Have previously received PB1046 or have a known allergy to the study drug or any of its components;

2. Participating in any other study and have received any other investigational medication or device within 30 days prior to screening or are taking part in a non-medication study which, in the opinion of the Investigator, would interfere with study compliance or outcome assessments;

3. Diagnosed with acute coronary syndrome (ACS) or an acute myocardial infarction (MI) within 3 months (90 days) of screening;

4. Canadian Cardiovascular Society (CCS) Class III or IV angina necessitating frequent use of as needed short acting nitroglycerin (e.g., sublingual);

5. Cardiac surgery or valvuloplasty within 3 months (90 days) prior to screening;

6. Cerebrovascular accident or transient ischemic attack within 3 months (90 days) prior to screening;

7. Sustained systolic blood pressure (SBP) < 110 mmHg and/or diastolic blood pressure (DBP) < 50 mmHg (confirmed by a duplicate seated reading) on at least 3 consecutive readings (self-monitored or office) prior to randomization or overt symptomatic hypotension;

8. Sustained resting heart rate >100 beats per minute (BPM) at screening (V1) or prior to randomization (confirmed on baseline telemetry monitoring);

9. History or evidence (documented on baseline telemetry monitoring) of clinically significant arrhythmias (uncontrolled by drug therapy or use of an implantable defibrillator), long QT syndrome or evidence of QT prolongation demonstrating non-paced QTcF > 460 ms (by ECG) prior to randomization (Subjects with QTcF > 460 ms due to electronic pacing by an implanted pacemaker/ICD device or a stable bundle branch block (BBB), may be enrolled);

10. Clinically significant renal dysfunction as measured by the estimated glomerular filtration rate (eGFR) of < 40 mL/min/1.73m2 as calculated by the CKD-EPI creatinine-cystatin C equation: eGFR = 135 x min (Scr/k, 1)-a × max(Scr/k, 1)-0.601 × min(Scys/0.8, 1)-0.375 × max(Scys/0.8, 1)-0.711 × 0.995age [× 0.969 if female] [× 1.08 if black] at screening, or a significant change in renal function between screening and baseline;

11. Clinically significant liver dysfunction as measured by: alanine aminotransferase >3.0 × the upper limit of normal (ULN), aspartate aminotransferase >3.0 × the ULN, or serum bilirubin ≥ 1.6 mg/dL at screening, or a significant change in liver function between screening and baseline;

12. Pregnant or lactating female subjects;

13. Known history of or active alcohol abuse or use of illicit drugs within 1 year prior to randomization;

14. Positive screening for human immunodeficiency virus antibodies, hepatitis B surface antigen, or hepatitis C virus antibodies;

15. Any major surgical procedure within 1 month (30 days) prior to screening or planned surgical procedure during the study period;

16. Other medical or psychiatric condition which, in the opinion of the Investigator, would place the subject at increased risk or would preclude obtaining voluntary consent/assent or would confound the secondary objectives of study.

2.1.4 Sample Size Justification

The sample size is not driven by inferential statistics. The sample size of four (4) (3 active and 1 placebo) for cohort 1 and eight (8) (6 active and 2 placebo) for subsequent cohorts for the 4 week multiple dose escalation study is set for qualitative investigation of toxicity as well as pharmacokinetics and to explore the potential of a pharmacodynamic response.

2.1.5 Randomization

Qualifying subjects will be randomly assigned via a central randomization system to receive either PB1046 or placebo. Only the unblinded study drug dispenser (USDD) at the study site will have access to the randomization assignment. However, in preparation of the Study Review Committee (SRC) meeting, when at least 75% of subjects assigned to a cohort have received at least 3 doses, independent study statistician, unblinded safety reviewer at BioTelemetry and pharmacokineticist (including PK analytical lab) will have access to the randomization codes for that cohort in order to prepare data summaries required for the study review meetings to assist in decisions regarding dose escalation. Displays will be provided to the committee in a blinded and partially unblinded (group) manner.

2.1.6 Scheduled Procedures and Flow Chart

SCHEDULE OF ACTIVITIES

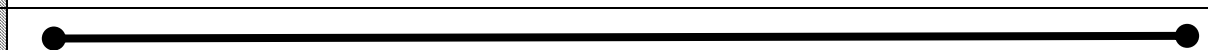
Week/Description	Blood Volume	Screen	Week -1	Week 1/ Dose 1	Week 1/ Daily Visits	Week 2/ Dose 2	Week 3/ Dose 3	Week 4/ Dose 4	Week 4/ Daily Visits	Week 5/ Daily Visits	Week 8/ Safety	Week 12/ Immuno-genicity
Visit Number		**V1**	**V2**	**V3**	**V4-V7**	**V8**	**V9**	**V10**	**V11-V14**	**V15-18**	**V19 or ET**	**V20**
Study Day		**-60**	**-7 (-10 to -7)**	**0**	**1, 2, 3 and 5**	**7**	**14**	**21**	**22, 23, 24 and 26**	**28, 29, 30, and 31**	**49 ± 3 days**	**77 ± 3 days**
Test *Protocol Reference*	**ml/ test**	**Number of Tests**										
Adverse Events *Refer to Section:**Error! eference source not found.***			1	1	4	1	1	1	4	4	1	
Concomitant Medications *Refer to Section:**Error! eference source not found.***		1	1	1	4	1	1	1	4	4	1	
Consent *Refer to Section **Error! Reference ource not found.***		1										
Demographics *Refer to Section **Error! Reference ource not found.***		1										
Directed Physical Examination *Refer to Section **Error! Reference ource not found.***			*As needed only for Evaluation of Adverse Events*									
Dosing (in office) *Refer to Section **Error! Reference ource not found.***				1		1	1	1				

Week/Description	Blood Volume	Screen	Week -1	Week 1/ Dose 1	Week 1/ Daily Visits	Week 2/ Dose 2	Week 3/ Dose 3	Week 4/ Dose 4	Week 4/ Daily Visits	Week 5/ Daily Visits	Week 8/ Safety	Week 12/ Immuno-genicity
Visit Number		**V1**	**V2**	**V3**	**V4-V7**	**V8**	**V9**	**V10**	**V11-V14**	**V15-18**	**V19 or ET**	**V20**
Study Day		**-60**	**-7 (-10 to -7)**	**0**	**1, 2, 3 and 5**	**7**	**14**	**21**	**22, 23, 24 and 26**	**28, 29, 30, and 31**	**49 ± 3 days**	**77 ± 3 days**
Test *Protocol Reference*	**ml/ test**	**Number of Tests**										
Dose Preparation (USDD) *Refer to Section* ***Error! Reference ource not found.***				1		1[k]	1[k]	1[k]				
Echocardiography - 2D with Doppler *Refer to Section* ***Error! Reference ource not found.***		1[g]	1[b,d]		1[v]				1[i]			
Electrocardiogram 12- lead *Refer to Section* ***Error! Reference ource not found.***		1		3[t,u]	4[o,n,u]	1[b]	1[b]	1[b,u]	4[o,n,u]	1[h,u]	1	
Height/Weight/BMI/BSA *Refer to Section* ***Error! Reference ource not found.***		1										
Histories – Medical, Targeted Medical, Surgical, Social and Medications *Refer to Section* ***Error! Reference ource not found.***		1										

Week/Description	Blood Volume	Screen	Week -1	Week 1/ Dose 1	Week 1/ Daily Visits	Week 2/ Dose 2	Week 3/ Dose 3	Week 4/ Dose 4	Week 4/ Daily Visits	Week 5/ Daily Visits	Week 8/ Safety	Week 12/ Immuno-genicity
Visit Number		**V1**	**V2**	**V3**	**V4-V7**	**V8**	**V9**	**V10**	**V11-V14**	**V15-18**	**V19 or ET**	**V20**
Study Day		**-60**	**-7 (-10 to -7)**	**0**	**1, 2, 3 and 5**	**7**	**14**	**21**	**22, 23, 24 and 26**	**28, 29, 30, and 31**	**49 ± 3 days**	**77 ± 3 days**
Test *Protocol Reference*	**ml/ test**	**Number of Tests**										
Inclusion/Exclusion *Refer to Sections* ***Error! Reference ource not found.****,* ***Error! Reference ource not found.****,* ***Error! Reference ource not found.****,* ***Error! Reference ource not found.****,* ***Error! Reference ource not found.****, and* ***Error! eference source not found.***		1	1	1[b]								
Injection Site Assessment *Refer to section* ***Error! Reference ource not found.***				1[a]	4[f]	1[b]	1[b]	1[b]	4[f]	4[f]	1	
LAB – Alcohol and drug screen (urine)[q] *Refer to Section* ***Error! Reference ource not found.***	NA	1	1	1[b]		1[b]	1[b]	1[b]				

Week/Description	Blood Volume	Screen	Week -1	Week 1/ Dose 1	Week 1/ Daily Visits	Week 2/ Dose 2	Week 3/ Dose 3	Week 4/ Dose 4	Week 4/ Daily Visits	Week 5/ Daily Visits	Week 8/ Safety	Week 12/ Immuno-genicity
Visit Number		**V1**	**V2**	**V3**	**V4-V7**	**V8**	**V9**	**V10**	**V11-V14**	**V15-18**	**V19 or ET**	**V20**
Study Day		**-60**	**-7 (-10 to -7)**	**0**	**1, 2, 3 and 5**	**7**	**14**	**21**	**22, 23, 24 and 26**	**28, 29, 30, and 31**	**49 ± 3 days**	**77 ± 3 days**
Test *Protocol Reference*	**ml/ test**	**Number of Tests**										
LAB - NT-proBNP and hs-cTnT *Refer to Sections* ***Error! Reference ource not found.*** *and* ***Error! eference source not found.***	4.0	1[c]		1[b]	3[o]	1[b]	1[b]	1[b]	1[i]	1[h]	1	
LAB - Exploratory Biomarkers: microRNA *Refer to Section* ***Error! Reference ource not found.***	2.5			1[b]	3[o]	1[b]	1[b]	1[b]	1[i]	1[h]	1	
LAB- Exploratory Biomarkers:Other *Refer to Section* ***Error! Reference ource not found.***	8			1[b]	3[o]	1[b]	1[b]	1[b]	1[i]	1[h]	1	
LAB - Immunogenicity *Refer to Section* ***Error! Reference ource not found.***	3.5			1[b,s]		1[b]	1[b]	1[b]		1[h]	1	1
LAB – Lipids/FPG *Refer to Sections* ***Error! Reference ource not found.*** *and* ***Error! eference source not found.***	9	1[p]	1[p]								1	

Week/Description	Blood Volume	Screen	Week -1	Week 1/ Dose 1	Week 1/ Daily Visits	Week 2/ Dose 2	Week 3/ Dose 3	Week 4/ Dose 4	Week 4/ Daily Visits	Week 5/ Daily Visits	Week 8/ Safety	Week 12/ Immuno-genicity
Visit Number		**V1**	**V2**	**V3**	**V4-V7**	**V8**	**V9**	**V10**	**V11-V14**	**V15-18**	**V19 or ET**	**V20**
Study Day		**-60**	**-7 (-10 to -7)**	**0**	**1, 2, 3 and 5**	**7**	**14**	**21**	**22, 23, 24 and 26**	**28, 29, 30, and 31**	**49 ± 3 days**	**77 ± 3 days**
Test *Protocol Reference*	**ml/ test**	**Number of Tests**										
LAB - Pharmacokinetics *Refer to Section* ***Error! Reference ource not found.***	3.5			4[b,e]	4[o,n]	1[b]	1[b]	4[b,e]	4[o, n]	4[f]		
LAB - Safety Labs *(Hematology, chemistry UA, eGFR calculation) Refer to Sections* ***Error! Reference ource not found.*** *and* ***Error! eference source not found.***	12	1	1			1[b]	1[b]	1[b]		1[h]	1	
LAB - Serology *(HIV, HepB and Hep C) Refer to Section* ***Error! Reference ource not found.***	4	1										
Pregnancy Testing (females of CBP) *Refer to* ***Error! eference source not found.***	NA	1	1	1[q,b]		1[b,q]	1[b, q]	1[b, q]				

Week/Description	Blood Volume	Screen	Week -1	Week 1/ Dose 1	Week 1/ Daily Visits	Week 2/ Dose 2	Week 3/ Dose 3	Week 4/ Dose 4	Week 4/ Daily Visits	Week 5/ Daily Visits	Week 8/ Safety	Week 12/ Immuno-genicity
Visit Number		**V1**	**V2**	**V3**	**V4-V7**	**V8**	**V9**	**V10**	**V11-V14**	**V15-18**	**V19 or ET**	**V20**
Study Day		**-60**	**-7 (-10 to -7)**	**0**	**1, 2, 3 and 5**	**7**	**14**	**21**	**22, 23, 24 and 26**	**28, 29, 30, and 31**	**49 ± 3 days**	**77 ± 3 days**
Test *Protocol Reference*	**ml/ test**	**Number of Tests**										
Physical Examination *Refer to Section **Error! Reference ource not found.***		1		1[b,j]							1	
Registration *Refer to Section **Error! Reference ource not found.***			1									
Randomization *Refer to Section **Error! Reference ource not found.Error! Reference source not found.***				1								
SMBP[m] *Refer to Section **Error! Reference ource not found.***												
Telemetry - mobile[l] *Refer to Section **Error! Reference ource not found.***												
Telephone contact[r] *Refer to section **Error! Reference ource not found.***				1		1	1	1				

Week/Description	Blood Volume	Screen	Week -1	Week 1/ Dose 1	Week 1/ Daily Visits	Week 2/ Dose 2	Week 3/ Dose 3	Week 4/ Dose 4	Week 4/ Daily Visits	Week 5/ Daily Visits	Week 8/ Safety	Week 12/ Immuno-genicity
Visit Number		**V1**	**V2**	**V3**	**V4-V7**	**V8**	**V9**	**V10**	**V11-V14**	**V15-18**	**V19 or ET**	**V20**
Study Day		**-60**	**-7 (-10 to -7)**	**0**	**1, 2, 3 and 5**	**7**	**14**	**21**	**22, 23, 24 and 26**	**28, 29, 30, and 31**	**49 ± 3 days**	**77 ± 3 days**
Test *Protocol Reference*	**ml/ test**	**Number of Tests**										
Vital Signs – Orthostatic *Refer to Section* ***Error! Reference ource not found.***		1		1[b]	4[f]	1[b]	1[b]	1[b]	4[f]	4[f]	1	
Vital Signs - Seated *Refer to Section* ***Error! Reference ource not found.***		1		3[b,a]	4[f]	1[b]	1[b]	1[b,a]	4[f]	4[f]	1	
Weight *Refer to Section* ***Error! Reference ource not found.***				1[b]		1[b]	1[b]	1[b]			1	
Total Blood Volume 369 mL		29	21	35.5	57.5	33.5	33.5	44	28.5	44	39	3.5

a 1 and 3 hours post dose (± 15 minutes)
b Prior to dosing
c NT-proBNP only
d Can occur up to 2 weeks (14 days) prior to scheduled randomization but should be done before placement of the mobile telemetry device.
e 1 and 3 hours (± 10 minutes)
f Daily ± 2 hours from previous dose time.
g If an historical (routine care) echo was within 60 days prior to screening and EF ≤ 40%, the screening echo will not need to be repeated. However, the baseline echo must be done between Day -14 and Day -7 to confirm final study eligibility by central reader.
h Day 28 only (± 2 hours after last dose)
i Day 24 only ± 1 day (collect biomarker and echo on the same days)
j Abbreviated physical examination
k ± 2 hours from time of day of previously weekly dose
l Continuous monitoring from approximately 7 days prior to dosing (baseline) through 7 days after the last dose of study drug.
m Omron Blood Pressure Monitor with ComFit™ Cuff (Model BP785N) for use of self-monitoring of blood pressure at home starting approximately 7 days prior to randomization. Subjects will be required to collect blood pressure and heart rate using the same arm every morning and every evening.
n 120 hours (± 26 hours) after last dose from previous dose time.
o Completed at 24, 48, and 72 hours (± 1 hour) after last dose. When PK and ECG are required at a given time point, the ECG should be done within 30 minutes and BEFORE collection of PK sample.

p Only performed/analyzed at screening if subject was fasting for at least 8 hours otherwise they will only be reported at Visit 2 when subject was instructed to fast prior to visit.
q Local testing only.
r Should occur in evening (suggested time between 7 and 10 PM).
s Collect 2 x 3.5mL tubes at baseline.
t Prior to dosing, at approximately -60 minutes, -30 minutes, -15 minutes
u Triplicate measurement, approximately 1 minute apart
v 48 (-6 to +24 hours) hours after dosing

3 ENDPOINTS

3.1 SAFETY ENDPOINTS

- Treatment emergent adverse events (TEAEs), serious adverse events (SAE), severity and relationship to study drug of TEAEs, adverse events (AEs) leading to discontinuation from the study, and deaths
- Actual and change from Baseline in clinical laboratory results
- Actual and change from Baseline in vital signs
- Actual and change from Baseline in physical examinations
- Actual and change from Baseline in ECG findings (heart rate (HR), QTcF, PR interval, and QRS)
- Actual and change from Baseline in telemetry data

3.2 PHARMACOKINETIC ENDPOINTS

For patients with enough PK data, the following parameters will be estimated for PB1046 by non-compartmental analysis methods in Phoenix® WinNonLin® 6.4 or higher.

Dose 1	C_{max}	Maximum plasma concentration (ng/mL)
	T_{max}	Time of maximum concentration (h)
	$AUC_{(0-\tau)}$	Area under the plasma concentration-time curve for the 7 day dosing period, calculated by linear trapezoidal summation.
	$AUC_{(0-tmax)}$	Area under the plasma concentration-time curve from time zero (predose) to T_{max}, calculated by linear trapezoidal summation.
	$AUC_{(tmax-\tau)}$	Area under the plasma concentration-time curve from T_{max} to last measurable concentration of the 7-day dosing period, calculated by linear trapezoidal summation.
Dose 4	C_{max}	Maximum plasma concentration (ng/mL), obtained directly from the observed concentration versus time data
	t_{max}	Time of maximum concentration (h), obtained directly from the observed concentration versus time data
	λ_Z	The elimination rate constant, λ_Z, will be calculated as the negative of the slope of the terminal log-linear

	segment of the serum concentration-time curve. The slope will be determined from a plot of the natural log of the terminal serum concentrations against time; at least 3 terminal serum concentration time points, beginning with the final concentration ≥ LOQ, will be selected for the determination of λ_Z and the regression will have a coefficient of determination (r^2) ≥ 0.9000.
t½	$t½ = \frac{0.693}{\lambda z}$
$AUC_{(0-\tau)}$	Area under the plasma concentration-time curve for the 7 day dosing period, calculated by linear trapezoidal summation.
$AUC_{(0-tmax)}$	Area under the plasma concentration-time curve from time zero (predose) to T_{max}, calculated by linear trapezoidal summation.
$AUC_{(tmax-\tau)}$	Area under the plasma concentration-time curve from T_{max} to last measurable concentration of the 7-day dosing period, calculated by linear trapezoidal summation.
CL/F	Total serum clearance (CL), corrected for bioavailability (F) $CL/F = \frac{Dose}{AUC\ (0-\tau)}$
Vz/F	Volume of distribution (Vz) corrected for bioavailability (F) $Vz/F = \frac{Dose}{\lambda z\ x\ AUC\ (0-\tau)}$
Ctrough	Pre-dose serum concentrations

3.3 IMMUNOGENICITY ENDPOINTS

- Incidence and characteristics of the anti-drug antibody (ADA) immune response
- Relationship of ADA with clinical safety and efficacy
- Relationship of ADA with pharmacokinetics (PK) and, when relevant, pharmacodynamic biomarkers

3.4 EFFICACY ENDPOINTS

- Actual and change from Baseline in 2-D echocardiographic parameters
- Actual and change from Baseline in exploratory cardiac, anti-inflammatory, and anti-fibrotic biomarkers

4 ANALYSIS POPULATIONS

Safety and intent-to-treat (ITT) population: The Safety and Intent-to-Treat (ITT) population will be the same and defined as all subjects who receive at least one dose of the study drug.

Per-protocol (PP) Population: The Per-Protocol (PP) population will be a subset of the ITT population excluding subjects who have any major violation of the protocol.

Pharmacokinetic (PK) Population: The pharmacokinetic population will consist of all subjects that receive active study drug and have sufficient data for pharmacokinetic analysis.

5 GENERAL ASPECTS OF STATISTICAL ANALYSIS

5.1 General Methods

The proposed statistical analysis methodologies are in accordance with the principles outlined by the International Conference on Harmonization (ICH) E9 guidelines[1]. Subject listings of all data represented in the electronic case report form (eCRF) and other vendor sourced assessments (eg, Diary) will be provided. Measurements from subjects excluded from the predefined analysis populations or extra measurements (such as unscheduled or repeat assessments) will not be included in summary tables, unless specified otherwise, but will be included in the subject listings. In general, the subject listings will be sorted by cohort, subject number, and assessment date (and time), if applicable. For summary tables, data will be presented by treatment group with placebo pooled.

Unless otherwise specified, continuous/quantitative variables will be summarized using descriptive statistics, which will include the number of subjects with data to be summarized (n), mean, standard deviation (SD), median, minimum, and maximum. The same number of decimal places as in the raw data will be presented when reporting the minimum and maximum, 1 more decimal place than in the raw data will be presented when reporting the mean and median, and 2 more decimal places than in the raw data will be presented when reporting the SD.

All categorical/qualitative data will be presented using frequency counts and percents. The total number of subjects in the treatment group overall (N) will be used as the denominator for percentage calculations, unless stated otherwise in the table shell. All percents will be presented as 1 decimal point, unless otherwise specified. Percents equal to 100 will be presented as 100% and percents will not be presented for zero frequencies.

All statistical tests will be 2-sided and have an associated α-level=0.05 unless mentioned otherwise. Confidence limits will be 2-sided and calculated with a 95% confidence coefficient, unless stated otherwise.

All statistical analyses will be done using SAS® (SAS Institute, Cary, NC) statistical software version 9.1.3 or higher.

5.2 Key Definitions

5.2.1 Baseline Values

For the safety, immunogenicity, and efficacy parameters assessed at the scheduled study visits, the baseline value is defined as the last non-missing measurement or assessment prior to the first dose of study drug.

5.2.2 Study Day

Subjects will be randomized and the first dose of randomized medication will be administered on Day 0. The Study Day is calculated as Event Date – First Dose Date.

5.2.3 End of Study

The end of study is defined as the date of early termination or the date of last safety assessment. It will be obtained from the eCRF. The end of study value is defined as the last non-missing measurement or assessment on or before the end of study.

5.3 Data Handling

5.3.1 Missing Data

When Baseline is missing, screening data may be used to impute Baseline value. Other missing data will not be imputed.

5.3.2 Per Protocol Time Points Vs Actual Time Points

Actual blood sampling times will be used unless mean plasma concentrations is required, in which case, per protocol times will be used. Details on PK data handling will be included in the PK section in the Appendix.

5.3.3 Repeated and Unscheduled Visit

The principle of 'last observation priority' will be used to handle the situation of repeated visit. Note that repeated visit is different from unscheduled visit, which can be identified from eCRF. The data will be updated base on the following rule:

- If a variable (except dates and all lab transferred data) is measured/collected at the repeated visit, the value of that variable will be replaced with the value from the repeated visit.
- Dates and Lab transferred data will be determined case by case based on its medical meaning.
- If a variable is not collected at the repeated visit, then the value from the original visit will be kept intact.
- Data from unscheduled visit will only be used in the analysis if specific visit has corresponding missing values and it is in a suitable time window. This change has to be reviewed and signed off by the medical monitor.

6 PLANNED ANALYSIS

6.1 Subject Disposition

Disposition will be summarized for each study period for all enrolled subjects. The number and percentage of subjects who complete or discontinue prematurely will be summarized by treatment group and overall. A listing of disposition will be provided for all subjects.

6.2 Protocol Violations

Protocol violations will be evaluated by the medical monitor and determined whether or not they are major protocol violations. Subjects with major protocol violations will be excluded from the PP population.

6.3 Demographics

Demographics and baseline characteristics will be listed and summarized by treatment group and overall for the safety population. Demographic characteristics will include age, sex, race, ethnicity, height, weight, body mass index (BMI), body surface area (BSA). Age (years) will be calculated as *INTEGER (*(Date of Informed Consent – Date of Birth)/365.25).

6.4 Medical History

Medical History, general and targeted, will be listed.

6.5 Dosing Information and Compliance

Information regarding dose cohort, dose level, volume, total dose and compliance be will be summarized by treatment group and study visit. Compliance is calculated as: compliance (%) = (actual volume injected) / (expected volume injected) x 100. All data will be listed as well.

6.6 Prior and Concomitant Medications

All medications and other treatments taken by subjects within 90 days prior to the screening period will be recorded on the eCRF. Prior medications are defined as medications taken prior to the first dose of study medication, regardless of whether they were continued or not after the first dose of study medication.
Concomitant medications are defined as medications that were taken on or after the first dose date, including those that started before the first dose date but continued into the Treatment Period.

Both prior and concomitant medications will be summarized by ATC code, preferred name and drug name. All data will be listed as well.

6.7 Efficacy Analysis

All efficacy analyses will be exploratory. The following efficacy variables will be considered in the efficacy analyses.

6.7.1 2-D Echocardiogram

2D Echocardiography is measured at screening, Day -7, Day 2 and Day 24. Day -7 value will be used as Baseline. The following echocardiographic parameters may be considered for evaluation. Change from baseline of these parameters will be calculated and all data will be listed by subject.

Blood pressure (BP) and heart rate (HR) (arterial peak, mean and diastolic blood pressure)
Left ventricular internal diameter in diastole (cm)
Left ventricular internal diameter in systole (cm)
Left ventricular wall thickness in diastole (cm; both septum and posterior wall)
Left ventricular outflow tract diameter in mid-systole (cm)
Left ventricular volumes (end-diastole and end-systole, in mL; apical views)
Left ventricular outflow tract flow-time velocity integral (by pulsed Doppler; in cm)
Ascending aorta peak flow velocity (by Continuous Wave Doppler; in m/s)
Transmitral flow velocity pattern (both by pulsed and Continuous Wave Doppler)
Doppler tissue imaging of tricuspid, septal and mitral annulus (velocity; in cm/s)
Left atrial volume (in mL/M2)

6.7.2 N-terminal Pro-B Type Natriuretic Peptide (NT-ProBNP)

NT-ProBNP measurement will be collected at Screening, day 0, day 1, 2, 3, 7, 14, 21, 24, and 28. Arithmetic and geometric mean of actual and change from Baseline NT-ProBNP will be summarized by treatment group and visits. Graphical presentation of mean change from Baseline NT-ProBNP vs study day will be included.

6.7.3 High Sensitivity Cardiac Troponin T Assay (hs-cTnT)

Hs-cTnT assessed at Screening, day 0, day 1, 2, 3, 7, 14, 21, 24, and 28. Arithmetic and geometric mean actual and change from Baseline NT-ProBNP will be summarized by treatment group and visits. Graphical presentation of mean change from Baseline hs-cTnT vs study day will be included.

6.7.4 Exploratory biomarkers

Exploratory biomarkers will be taken at day 0 prior to dosing, day 1, 2, 3, 7, 14, 21, 24, and 28. Samples taken at day 0, 14, and 28 are being evaluated. Change from baseline of these parameters will be calculated. Additional timepoints will be evaluated as warranted. This data will be provided by Sponsor but will not be incorporated into the eCRF system. Actual and change from Baseline of each biomarker value will be summarized by treatment group and visits. All data will be listed by subject.

Exploratory Biomarkers (list may not be all encompassing)	
Flow Cytometry	CD2, CD3, CD4, CD8, CD16, CD19, CD25, CD45, and CD26
3 plex kit	MMP-1, MMP-3, MMP-9 (matrix metalloproteinases)
	TGF-β1
	CRP (C-Reactive Protein)

	Galectin-3
10 plex kit	IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IFN-γ, and TNF α
	TIMP-1 (tissue inhibitor of metalloproteinase 1)
	ST2 (growth stimulation expressed gene 2)
	IL-33

6.8 Safety Analysis

6.8.1 Adverse Events (AE)

TEAEs are defined as any AEs that started on or after the first dose of study drug. All reported AEs (including non-TEAEs) will be listed, but only TEAEs will be summarized in tables.

An overall summary of TEAEs will be provided, by treatment group and overall, the number and percentage of subjects who reported:

- Subjects reporting at least one TEAE (n, %)
- Total number of TEAEs
- Subjects reporting at least one severe TEAEs (n, %)
- Total number of severe TEAEs
- Subjects reporting a TEAE related to study treatment (n, %)
- Total number of related TEAEs
- Subjects reporting a serious adverse event (SAE) (n, %)
- Total number of SAEs
- Subjects reporting dose limiting toxicity (DLT) (n, %)
- Total number of DLT
- Number of subjects who had a TEAE leading to study discontinuation
- Subjects who died (n, %)

A set of summary tables of TEAEs by SOC and PT will also be presented for the following categories.

- TEAEs
- SAEs
- Severe TEAEs
- Treatment-related TEAEs
- TEAEs leading to study discontinuation

In the summary tables subjects may be counted under multiple SOCs and PTs, but for each SOC and PT, subjects are only counted once. If a subject has the same AE

on multiple occasions, the highest severity (severe > moderate > mild) or drug relationship (definitely related > probably related > possibly related > unlikely related > not-related) recorded for the event will be presented as needed by the table summary of interest. If severity of AE is missing in the database, it will be programmed to be 'severe' for the counts in the summary table. If drug relationship is missing then the AE will be considered as 'related' for the summary table.

Listings will be provided for all AEs, SAEs, AEs leading to study discontinuation, and deaths.

6.8.2 Injection Site Reactions (ISRs)

ISRs are captured under AE domain but will be evaluated separately by severity and toxicity grade. The toxicity is graded as the following:

- Grade 1 – Mild
- Grade 2 – Moderate
- Grade 3 – Severe
- Grade 4 – Life threatening or disabling

ISRs will be summarized by PTs, toxicity grade. The following overall summary will be included in the beginning part of the table:

- Subjects reporting at least one ISR (n, %)
- Total number of ISRs
- Subjects reporting at least one severe (toxicity ≥ 3) ISR (n, %)
- Total number of severe ISRs
- Subjects reporting dose limiting toxicity (DLT) (n, %)
- Total number of DLT
- Number of subjects who had a ISR leading to study discontinuation
- Subjects who died (n, %)

6.8.3 ECG

ECG parameters will be imported directly from the electronic ECG database (BioTelemetry) based on the data transfer specifications.

Change from baseline will be calculated for each visit day (refer to schedule below). Baseline is defined as the mean of 9 pre-dose ECG collected at approximately -60 (T2, T3, T4). -30 (T5, T6, T7) and -15 (T8, T9, T10) minutes before dosing on Day 0.

Visit Description	Time Point	Data Handling
Screening	1	

Day 0 / Pre-Dose (-60 mins)	2, 3, 4	Baseline = mean of 9 measurements
Day 0 / Pre-Dose (-30 mins)	5, 6, 7	
Day 0 / Pre-Dose (-15 mins)	8, 9, 10	
Day 1	11, 12, 13	Mean of three measurements
Day 2	14, 15, 16	Mean of three measurements
Day 3	17, 18, 19	Mean of three measurements
Day 5	20, 21, 22	Mean of three measurements
Day 7	23	
Day 14	24	
Day 21	25, 26, 27	Mean of three measurements
Day 22	28, 29, 30	Mean of three measurements
Day 23	31, 32, 33	Mean of three measurements
Day 24	34, 35, 36	Mean of three measurements
Day 26	37, 38, 39	Mean of three measurements
Day 28	40, 41, 42	Mean of three measurements
Day 49 (± 3 days)	43	
Unscheduled	99	

The following ECG parameters will be recorded: heart rate (bpm), PR interval (msec), QRS interval (msec), QT interval (msec), and corrected QT (QTc) intervals (msec). QT will be corrected for heart rate using Fridericia's (QTcF) interval and Bazett's (QTcB).

For each ECG parameter, the observed value and change from Baseline value will be summarized using descriptive statistics by study visit and treatment group for all subjects in the safety population.

In addition, counts and percentages of subjects with outlying QT/QTc values (absolute value >500 msec, increase from Baseline value of >30 or >60 msec, or 15% or more change from Baseline) based on ICH Guidance E14[2] will be presented by study visit and treatment group.

Changes of QTc vs serum exposure from PK data might also be investigated.
Graphical presentation of actual and change from Baseline in ECG parameters over time will be included.

All ECG data will be included in subject listings.

6.8.4 Telemetry

Telemetry data will be listed by subjects.

6.8.5 Vital Signs

Vital signs include blood pressure (seated and orthostatic), heart rate, respiratory rate, and body temperature. Orthostatic blood pressure will be assessed at screening, prior to each dosing and daily on Week 1, Week 4 and Week 5. The rest of the vital signs will be assessed at screening, prior to dosing of each week, 1hr and 3 hrs post dosing of Week 1 and Week 4, daily on the rest of the days of Week 1 and Week 4, and daily on Week 5.

For each vital sign parameter, the observed value and change from Baseline value will be summarized using descriptive statistics by study visit and treatment group. Percent change from Baseline in seated blood pressure will also be summarized. Mean of the actual and change from Baseline seated and orthostatic blood pressure will be plotted against study days to visualize the changes over time. % change from Baseline in seated blood pressure vs study days will also be plotted.

All vital signs data will be presented in the subject listings.

6.8.6 Physical Examination

Physical examination includes but not limited to examination of general appearance, skin, eyes, ears, nose, throat, neck/thyroid, lungs, heart, upper/lower extremities, lymph nodes, abdomen, musculoskeletal system and neurological system. Clinically significant findings will be recorded as an adverse event.

Weight is measured on Screening, prior to each dosing from Week 1 to Week 4, and on Week 8. Change from Baseline weight value will be calculated.

Physical examination and weight results will be presented in a data listing.

6.8.7 Laboratory Evaluations

Laboratory tests include safety labs (Hematology, Chemistry, Urinalysis, eGFR), Serology (HIV, HepB and Hep C), lipid, drug and alcohol test, pregnancy test (urine and serum).

Hematology, Chemistry, eGFR results and their changes from Baseline value will be summarized by treatment group and analysis visit using descriptive statistics. The Baseline value and change from Baseline value at each visit will be provided for all subjects in the safety population.

The laboratory test result will be reported as “High”, “Low”, or “Normal” with respect to relevant reference ranges.
All laboratory data will be listed. Laboratory test results falling out of the normal range will be marked as high or low in the listings.

6.8.8 Self-monitored Blood Pressure (SMBP)

SMBP will be entered in the eCRF from the paper source subject diary. Missing blood pressure values will not be imputed. SMBP is collected by patient once in the morning and once in the evening. Mean of the actual and change from Baseline SMBP for morning or evening will be plotted against study days to visualize the changes over time.

6.9 Pharmacokinetics (PK)

PK assessments will be performed on serum concentrations from subjects receiving PB1046. All non-compartmental pharmacokinetic calculations and generation of individual subject serum concentration vs. time graphs will be performed using Phoenix WinNonlin 6.4 or higher. Graphs of mean serum concentrations vs. time and other in-text figures will be prepared using Phoenix WinNonlin 6.4 or higher. Compartmental modeling will be done using Phoenix WinNonlin 6.4 or higher, or Phoenix NLME 1.3 or higher, or NONMEM 7.3 or higher. Exploratory pharmacokinetic/pharmacodynamics analyses will be done using appropriate software.

6.9.1 Measurements and Collection Schedule

Blood samples to determine serum concentrations of PB1046 will be collected as follow:

-Week 1: 0 (pre-dose), 1, 3, 24, 48, 72, and 120 hours post-dose,
-Week 2 and 3: 0 (pre-dose) hour,
-Week 4: 0 (pre-dose), 1, 3, 24, 48, 72, 120, 168, 192, 216, and 240 hours post-dose.

The acceptable windows for collection of blood samples for PK are the following: ± 10 minutes (sampling at 1 and 3 hours post-dose), ± 1 hour (sampling at 24, 47, and 72 hours post-dose), ± 26 hours (sampling at 120 hours post-dose).

6.9.2 Pharmacokinetic Data Handling

PK parameters from serum concentrations for PB1046 will be calculated using a non-compartmental approach based on the concentration vs time data. The parameters listed in the table below (but not limited to) will be obtained, as data permit.

The PK parameters will be provided according to PB1046 dose levels. Subjects for whom there is insufficient data to calculate the PK parameters will have available data included in the concentration tables with descriptive statistics only. Only serum concentrations equal to or greater than the validated lower limit of the assay (LOQ) will be used in the pharmacokinetic analysis. Serum concentrations <LOQ will be taken as 0 for the calculation of the descriptive statistics for serum concentrations at each sampling time. For the calculation of the PK parameters, serum concentrations < LOQ that occur from pre-dose to the first concentration ≥ LOQ will be taken as 0 and those that occur thereafter will be taken as missing.

Actual blood sampling times relative to dosing will be used in the computation of PK parameters. A minimum of 3 descending concentration-time points, excluding the C_{max}, above the lower limit of quantification (LLOQ) will be used in the estimation of the elimination rate constant (λ_z) and the regression will have a coefficient of determination (r^2) ≥ 0.9000, for the determination of the elimination half-life ($t_{1/2}$). For those subjects for whom λ_z cannot be estimated, λ_z, $t_{1/2}$, CL/F, and V_z/F will be assigned a value of missing.

Unless otherwise specified below, missing sampling or concentration values will not be imputed, but left missing in the calculation of derived PK parameters. If the actual sampling time is missing, but a valid concentration value has been measured, the scheduled protocol time will be used for the calculation of derived PK parameters.

On a case by case basis, it may be necessary to exclude individual PK concentration values for the calculation of derived PK parameters because they are erroneous, abnormal or appear implausible to the pharmacokineticist in charge of the analysis. Any excluded data will be flagged in the individual data listings. The reason for exclusion will also be documented. If the exclusion has a meaningful impact on the overall interpretation of the results, then it will be discussed in the PK report.

6.9.3 PK Analysis

Actual blood sampling times will be used in the generation of individual concentration-time profiles. Per protocol times will be used to calculate mean serum concentrations for tabular and graphical displays.

Pharmacokinetic analysis will be carried out for the PK population. Individual PK parameters will be listed. Descriptive statistics (number of non-missing observations (N), arithmetic mean, SD, minimum, median, maximum, coefficient of variation (CV%), geometric mean and geometric CV%) will be used to summarize the calculated PK parameters by dose levels and dosing weeks.

Individual concentration profiles with actual blood sampling times will be listed. Descriptive summary statistics (N, arithmetic mean, SD, minimum, median, maximum, CV%, geometric mean and geometric CV%) will be used to summarize the concentration profiles by dose levels and dosing weeks.

Individual time-concentration graphs will be provided for each subject in both linear and semi-log scales. Mean time-concentration graphs will be provided for each dose levels in both linear and semi-log scales to provide visual examination of PK profiles after multiple doses of PB1046 and to facilitate steady state and accumulation assessments. Descriptive statistics of the pre-dose serum concentrations (Ctrough) measured from Weeks 1 through 4 and the concentration 7 days after the last dose and the plot of individual/mean Ctrough-time profiles will be presented to facilitate the steady-state assessment.

6.9.4 Pharmacokinetic Parameters — Compartmental Modeling

If the graphical presentations of the individual subject serum concentrations vs. time suggest that a compartmental model may be consistent with the data, then an appropriate model will be fit to each subject's complete set of data. The specific model will be determined after examination of the graphs and/or a statistical comparison of the fit of various models, as data permit.

6.10 Immunogenicity (Antibody)

Immunogenicity results will be presented in tabular, text, or graphical form as appropriate. The extent of analysis for evaluating the clinical relevance of ADA-positive samples depends upon the phase of development, sample size, and ADA incidence. Thus the type and extent of analysis will be driven by sound scientific judgement and justified in the CSR[4].

Counts and percentages of subjects will be classified by their ADA status and treatment group.

In a post-hoc analysis, immunogenicity data will be assessed to determine the clinical relevance. The relationship to anticipated or relevant adverse events, such as the occurrence of injection site reactions, in antibody positive and antibody negative PB1046 treated subjects will be evaluated. In addition to the potential impact on safety, subjects testing positive for anti-drug antibodies may be evaluated for any impact on PK and clinical efficacy.

6.11 Deviations From the Protocol Specified Analysis

There are no deviations from the protocol-specified analysis.

7 REFERENCE LIST

1. ICH Harmonised Tripartite Guideline “E9 Statistical principles for clinical trials” http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E9/Step4/E9_Guideline.pdf. Accessed May 15, 2015.

2. ICH Guidance "E14 Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs” **Error! Hyperlink reference not valid.**http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm073153.pdf. Accessed May 04, 2015

3. ICH Guidance “E3 Structure and Content of Clinical Study Reports”. http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E3/E3_Guideline.pdf. Accessed May 04, 2015.

4. Shankar G, Arkin S, Cocea L, Devanarayan V, Kirshner S, Kromminga A, Quarmby V, Richards S, Schneider CK, Subramanyam M, Swanson S, Verthelyi D, Yim S. Assessment and Reporting of the Clinical Immunogenicity of Therapeutic Protein and Peptides – Harmonized Terminology and Tactical Recommendations. AAPS J. 2014 Jul;16(4):658-73.

8 LIST OF PLANED LISTING, TABLE AND FIGURES

Please see the Appendix 1.

Numbering of TLFs will follow ICH E3 guidance[3], unless otherwise specified. Note that this is the planned Table of Content, based on the actual data, table numbers, table names and structures may change.

9 SOFTWARE DOCUMENTATION

PC SAS – Windows version 9.1.3 or higher.

PC R - Windows version 12.0 or higher.

WinNonlin - Professional Version 5.2 or higher.

Excel - Microsoft Excel 2007 or higher.

10 APPENDIX

1. Statistical Analysis Plan V1 - Appendix 1 Table of contents V1.xlsx