CLINICAL STUDY PROTOCOL

A phase 1b study investigating the safety, tolerability and efficacy of PP353 in the treatment of patients with chronic low back pain associated with vertebral body endplate bone oedema (Modic 1)

Development Phase:	Phase 1b
Product Name:	PP353
IND Number:	n/a
EudraCT number:	2018-004488-30
Universal Trial Number:	U1111-1257-2567
Title:	A phase 1b study investigating the safety, tolerability and efficacy of PP353 in the treatment of patients with chronic low back pain associated with vertebral body endplate bone oedema (Modic 1)
Sponsor:	Persica Pharmaceuticals Ltd. 7 Denne Hill Business Centre, Womenswold, Canterbury, Kent, CT4 6HD
Protocol version and date:	Version 1.0 - 08 February 2019
Date amended:	Amendment 1 – 29 April 2019
Date amended:	Amendment 2 – 07 June 2019
Date amended:	Amendment 3 – 06 March 2020
Date amended:	Amendment 3.1 – 05 August 2021
Date amended:	Amendment 4 – 09 August 2021
Date amended:	Amendment 5 – 20 July 2022
Date amended:	Amendment 6 – 14 Mar 2024
Study Number	Persica 002

CONDUCT

In accordance with the ethical principles that originate from the Declaration of Helsinki and that are consistent with International Conference on Harmonisation (ICH) guidelines on Good Clinical Practice (GCP) and regulatory requirements as applicable.

Signatures

Signature of Sponsor Representative

Title: A phase 1b study investigating the safety, tolerability and efficacy of PP353 in the treatment of patients with chronic low back pain associated with vertebral body endplate bone oedema (Modic 1)

Sponsor Representative:	Persica Senior Management
Name:	[redacted]
This Clinical Study Protocol has been reviewed and approved by Persica Senior Management	
[redacted]	[redacted]
Date:	21-Mar-2024

Sponsor Representative:	Medical Consultant
Name:	[redacted]
[redacted]	[redacted]
This Clinical Study Protocol has been reviewed and approved by Sponsor Medical Consultant	
[redacted]	[redacted]
Date:	22-Mar-2024

Signature of the Clinical Pharmacologist

Title: A phase 1b study investigating the safety, tolerability and efficacy of PP353 in the treatment of patients with chronic low back pain associated with vertebral body endplate bone oedema (Modic 1)

Name:	[redacted]
Affiliation:	[redacted]
This Clinical Study Protocol has been reviewed and approved by the clinical pharmacologist in order to ensure that the protocol and any amendments (if applicable) cover all relevant pharmacokinetic matters clearly and accurately, using technical terminology as appropriate.	
[redacted]	[redacted]
Date:	22-Mar-2024

Signature of the Statistician

Title: A phase 1b study investigating the safety, tolerability and efficacy of PP353 in the treatment of patients with chronic low back pain associated with vertebral body endplate bone oedema (Modic 1)

Name:	[redacted]
Affiliation:	[redacted]
This Clinical Study Protocol has been reviewed and approved by the statistician in order to ensure that the protocol and any amendments (if applicable) cover all relevant statistical matters clearly and accurately, using technical terminology as appropriate.	
[redacted]	[redacted]
Date:	22-Mar-2024

Signature of Investigator

Title: A phase 1b study investigating the safety, tolerability and efficacy of PP353 in the treatment of patients with chronic low back pain associated with vertebral body endplate bone oedema (Modic 1)

Name:

Affiliation:

Address:

I have read and understood all sections of the protocol entitled, "A phase 1b study investigating the safety, tolerability and efficacy of PP353 in the treatment of subjects with chronic low back pain associated with vertebral body endplate bone oedema (Modic 1).

I agree to supervise all aspects of the protocol and to conduct the clinical investigation in accordance with the Protocol Amendment 6, dated 14 Mar 2024, the International Conference on Harmonisation tripartite guideline E6(R2): Good Clinical Practice and all applicable government regulations. I will not make changes to the protocol before consulting with Persica Pharmaceuticals Ltd. or implement protocol changes without independent ethics committee approval except to eliminate an immediate risk to subjects. I agree to administer study treatment only to subjects under my personal supervision or the supervision of a sub-investigator.

I will not supply the investigational product to any person not authorised to receive it. Confidentiality will be protected. Subject identity will not be disclosed to third parties or appear in any study reports or publications.

I will not disclose information regarding this clinical investigation or publish results of the investigation without authorisation from Persica Pharmaceuticals Ltd.

Signature:

Date:

Protocol History

Protocol History **Persica Pharmaceuticals Ltd** – Persica 002			
Document	**Version No Issue Date**	**Previous text**	**Revised text**
Clinical Study Protocol	V1.0_08/Feb/2019	-	NA
Clinical Study Protocol amendment	Amendment 1_29/Apr/2019	Refer to Appendix F	
	The Sponsor has decided that this amendment is a **substantial** amendment. **Justification:** This protocol amendment removes the requirement for general anaesthesia to be used during drug administration and clarifies that the subject should be alert, conscious and responsive during spinal needle insertion past the nerve root. It further amends the name of the associated manual from Theatre Procedure Manual to Procedure Manual. The anaesthetic practices used for intradiscal injections may be different at different sites and combinations of short and long acting local anaesthetics, with or without sedation or general anaesthesia, can be safely used to perform the injection. Additionally, the potential for nerve root damage should be avoided. In order to accommodate different anaesthetic practices at the different sites, possible approaches will be articulated in the Procedure Manual.		
Clinical Study Protocol amendment	Amendment 2_07/June/2019	Refer to Appendix F	
	The Sponsor has decided that this amendment is a **substantial** amendment. **Justification:** In additional to administrative changes and inclusion of points of clarification, this protocol amendment adds tapentadol as a prohibited concomitant treatment for the duration of the study, specifies that hospitalization on the day of study drug administration for logistical reasons will not be reported as a SAE and disallows the use of linezolid during the intervention period.		
Clinical Study Protocol amendment	Amendment 3_06/March/2020	Refer to Appendix F	
	The Sponsor has decided that this amendment is a **substantial** amendment. **Justification:** In additional to administrative changes and inclusion of points of clarification, this protocol amendment incorporates changes to the inclusion/exclusion criteria and the stopping rules. It also allows conduct of a suitability MRI in cases where a standard-of care MRI is not available.		
Clinical Study Protocol amendment	Amendment 3.1_05/08/2021	Refer to Appendix F	
	The Sponsor has decided that this amendment is a **non-substantial** amendment. **Justification:** As allowed in previous protocol versions, the schedule of activities for Part B (intervention period) has been amended following completion of Part A. Foot notes relating to Schedule of Activities (2 injections) have been moved into the Schedule of Activities table (notes column) along with other points of clarification.		
Clinical Study Protocol amendment	Amendment 4_09/08/2021	Refer to Appendix F	
	The Sponsor has decided that this amendment is a **substantial** amendment. **Justification:** In additional to administrative changes and inclusion of points of clarification, this protocol amendment incorporates the following changes; removal of overnight stay, simplification of the schedule of assessments, removal of restrictions to concomitant medications, changes to inclusion/exclusion criteria, addition of average LBP NRS questionnaire, DCE MRIs are advised but no longer mandatory, confirm dosing days on Day 1 and 5 (±1 day), increase number of subjects in Part B from 34 to 40, add the possibility analysing for the excipients of PP353 in PK samples and to amend placebo group dosing schedule.		
Clinical Study Protocol amendment	Amendment 5_20/07/2022	Refer to Appendix F	
	The Sponsor has decided that this amendment is a **substantial** amendment. **Justification:** In addition to administrative changes and inclusion of points of clarification, this protocol amendment incorporates the following changes: increase screening window from 28 to 56 days and PK sampling requirements will be reduced after 1st 12 subjects in Part B.		
Clinical Study Protocol amendment	Amendment 6_14/03/2024	Refer to Appendix F	
	The Sponsor has decided that this amendment is a **substantial** amendment. **Justification:** This protocol amendment incorporates the following changes: To test available baseline PK samples for cytokines.		

Table of Contents

List of Abbreviations and Definitions of Terms

Abbreviation	Definition
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
Anti-HCV	Hepatitis C antibodies
API	Active pharmaceutical ingredient
AST	Aspartate aminotransferase
AUC	Area under the concentration time curve
AUC_{24h}	Area under the concentration time curve from zero to 24 hours
$AUC_{0-\infty}$	Area under the concentration time curve from time zero to infinity
AUC_{τ}	Area under the concentration time curve calculated over the dosing interval at steady-state
AUC_{0-t}	Area under the concentration time curve from time zero to time t (the last observable concentration)
CHMP	Committee for Medicinal Products for Human Use
CLBP	Chronic low back pain
CL_{ss}/F	Apparent clearance of drug from plasma at steady-state
C_{max}	Maximum (peak) plasma concentration
$C_{max,ss}$	C_{max} during the dosing interval at steady-state
CI	Confidence intervals
CRA	Clinical research associate
CRO	Contract research organisation
CRF	Case report form
DCE-MRI	Dynamic contrast enhanced magnetic resonance imaging
DDIs	Drug-Drug Interactions
EC	Ethics committee
ECG	Electrocardiogram
eCRF	Electronic case report form
eGFR	Estimated glomerular filtration rate
EDC	Electronic data capture
EoS	End of study
ES	Enrolled set
ET	Early termination
FAS	Full analysis set
FDA	Food and Drug Administration
FIH	First in human
FSH	Follicle-stimulating hormone
Gd	Gadolinium
GCP	Good Clinical Practice
GGT	Gamma glutamyl transferase
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HBsAg	Hepatitis B surface antigen
HIV	Human immunodeficiency virus
IB	Investigator's brochure
ICF	Informed consent form
ICH	International Council for Harmonisation
ICMJE	International Committee of Medical Journal Editors

Abbreviation	Definition
IEC	Independent ethics committee
IMP	Investigational medicinal product
IND	Investigational new drug
IRT	Interactive response technology
i.v.	Intravenous
kg	Kilogram
L1	First lumbar vertebra
LBP	Low back pain
LBP NRS	Low back pain numeric rating scale
LC-MS/MS	Liquid chromatography-mass spectrometry/mass spectrometry
LPLV	Last patient last visit
LS	Least squares
MAD	Mutual acceptance of data
MAOI	Monoamine oxidase inhibitors
MDRD	Modified Diet in Renal Disease study
mg	Milligram
MIC	Minimum inhibitory concentration
mL	Millilitres
MMRM	Mixed model for repeated measures
MRI	Magnetic resonance imaging
NRS	Numeric rating scale
NYHA	New York Heart Association
ODI	Oswestry Disability Index
OECD	Organisation for Economic Co-operation and Development
OTC	Over the counter
PCR	Polymerase chain reaction
PK	Pharmacokinetic(s)
PPS	Per protocol set
PSMT	Persica senior management team
QTcB	Corrected QT interval using Bazett's method
QC	Quality Control
RBC	Red blood cell
RCT	Randomised controlled trial
RMDQ	Roland Morris Disability Questionnaire
RS	Randomised set
S1	First sacral vertebra
SAE	Serious adverse event
SD	Standard deviation
SmPC	Summary of product characteristics
SRC	Safety review committee
SS	Safety set
SSRI	Selective Serotonin Reuptake Inhibitor
STIR	Short-TI inversion recovery
SUSAR	Suspected unexpected serious adverse reaction
$t_{1/2,z}$	Terminal-phase elimination half-life
t_{max}	Time to maximum (peak) plasma concentration
ULN	Upper limit of normal
WBC	White blood cell

Synopsis

Name of Sponsor/Company: Persica Pharmaceuticals Ltd.
Name of Investigational Product: PP353
Title of Study: **A phase 1b study investigating the safety, tolerability and efficacy of PP353 in the treatment of patients with chronic low back pain associated with vertebral body endplate bone oedema (Modic 1)**
Study centre(s): Multi-centre
Development Phase: 1b
METHODOLOGY This is a two-part study, each of 12 months' duration, in subjects with chronic low back pain (CLBP) associated with vertebral body endplate bone oedema (Modic 1), as detected by MRI. In both parts of the study, the subject population will not have been previously treated with antimicrobial agents for their back pain. Part A of the study is open label to evaluate the safety, tolerability and pharmacokinetics (PK) of PP353 in the above subjects. Part B of the study is a randomised, placebo-controlled, investigator- and subject-blinded, third-party unblinded study to evaluate the safety, tolerability and efficacy of two administrations of PP353 given approximately 4 days apart in the above subjects.
<u>Part A</u> Open label cohort of up to six subjects to study the safety, tolerability and PK of PP353 after a single intradiscal injection. • 3 + 3 subjects The aim of Part A is to determine the injection regimen for Part B and to establish the tolerability of a single injection of PP353. The first three subjects will receive a single injection of up to 3 mL of PP353 delivered directly into the intervertebral disc under contrast guidance. Systemic (blood) sampling will be performed over the next 10 days to establish the PK of PP353. The subjects will be dosed sequentially with a gap of at least 14 days between each subject being injected. Following a review of the safety and PK data for the first three subjects, a decision will be made by the safety review committee (SRC) as to whether the PK data are consistent enough to determine a choice of regimen for Part B. If the PK data are not consistent (if the variability is too high to provide a reliable PK profile and there is not enough information to confirm the PK sample timepoints in Part B), then a further three subjects can be enrolled into Part A. The second set of three subjects can be dosed sequentially or in parallel depending on the recruitment rate. The timing of the

PK samples for the second set of three subjects (Subjects 4–6) may be altered following analysis of the first three subjects in order to optimise PK understanding.

Subjects will be followed for 12 months post dose to evaluate the safety, and tolerability of PP353. The clinical benefit of PP353 will also be evaluated as an exploratory endpoint in Part A of this study.

Part B

Randomised, investigator- and subject-blinded, and third-party (pharmacist and drug administrator) unblinded, placebo-controlled study of up to 40 subjects, to study the safety, tolerability and efficacy of PP353 in treating subjects with CLBP associated with vertebral body endplate bone oedema detected on MRI imaging.

- 20 subjects on active
- 20 subjects on placebo

Part B consists of two arms, comparing active versus placebo for safety, tolerability and efficacy of PP353. Placebo will consist of sham injections.

The dosing regimen in Part B was determined by the PK observed in Part A and will consist of two intradiscal injections providing approximately 8 days exposure to PP353 within the intervertebral disc, as inferred from plasma exposure. Subjects on the active arm will be treated with two intradiscal injections administered on Day 1 and then Day 5. Subjects on placebo will receive two sham injections, with the skin pierced and the needle penetrating the deep fascia, stopping 2 to 3 cm short of the intervertebral disc.

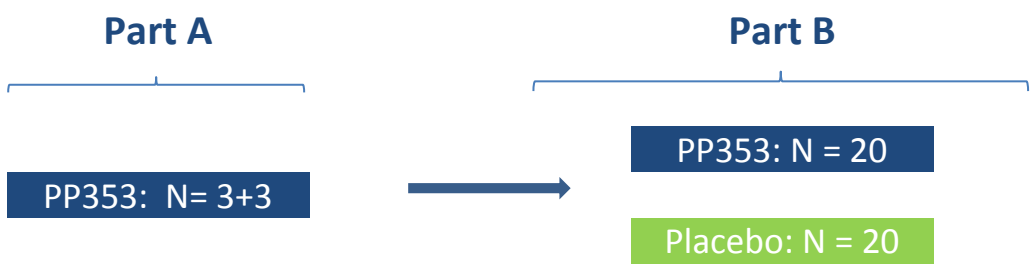
Subjects will be followed for 12 months post dose to evaluate the safety, tolerability and efficacy of PP353.

As more than one intradiscal injection is required in Part B, a sentinel pair (one subject on active and one subject on placebo) will be dosed initially, followed by the rest of the cohort no sooner than 14 days after the last injection of the sentinel pair.

Study Design

The study overview is presented in Figure 1.

Figure 1: PP353 Study Overview



Description of Part A and B

Part A:

Up to six subjects with CLBP for longer than 6 months and vertebral body endplate bone oedema who, at screening, have had an inadequate response to current standard of care. An inadequate response to current standard of care is defined as having a residual pain score of ≥ 4 on chronic pain medication and ≥ 6 if not on chronic pain medication on the Low Back Pain Numeric Rating Scale (LBP NRS) and a Roland Morris Disability Questionnaire (RMDQ-23) score of ≥ 9.

Subjects will have an MRI, including macrocyclic gadolinium-enhanced dynamic contrast MRI (DCE-MRI) scan at baseline, 6 months' and 12 months' participation (or early termination) in the study. The MRI protocol will be designed to have T1 and T2 for assessment of MODIC changes, sequences measuring inflammation (short-T1 inversion recovery [STIR] or DCE-MRI) and sequences measuring spine lesions (DIXON).

Subjects will remain in hospital for an overnight period after the injection and will remain in a recumbent position (on bed rest) for the first 8 hours after administration. Subjects will be advised to undertake only modest activity levels that do not provoke pain for the first 14 days following injection, and only engage in activity within pain limits thereafter.

Part B:

Up to 40 subjects with CLBP for longer than 6 months and vertebral body endplate bone oedema who have had an inadequate response to current standard of care, defined as for Part A. The two arms consist of:

Arm 1: active

Twenty subjects treated with intradiscal injections of PP353. Subjects will have two injections and will then be followed for 12 months to establish safety, tolerability and efficacy of PP353.

Arm 2: placebo

Twenty subjects treated with two placebo injections. Subjects will have the same number of injections as in Arm 1 to match the active treatment arm and maintain the blind. Subjects will be followed for 12 months to establish safety, tolerability and efficacy of PP353.

All participants in Part B will have an MRI exam, at baseline, 6 months and at 12 months; DCE-MRI sequences should be done on all subjects where possible. In cases where the DCE-MRI procedure cannot be conducted by the site or the subject is unable to tolerate the procedure, the DCE-MRI sequence can be omitted. However, all other sequences should be performed. If the baseline DCE-MRI has not been conducted, then DCE-MRI should not be performed at the 6 or 12 month visit. The acquisition of a DCE-MRI sequence at 6 months will be decided by the SRC and is dependent on the ability to visualise and quantify inflammatory changes in the spine, which provide scientific and clinical value to the subject

assessment. The decision will be based on the available observations made in Part A of the study and available Part B screening MRIs.

Subjects in Part B must remain in hospital and have limited activity for at least 8 hours after each injection in order to maximise the opportunity for the study medication to remain within the intervertebral disc space; they should remain lying down on bed rest, e.g. recumbent, semi-recumbent or on their side, for the first 4 hours and then have limited activity for the subsequent 4 hours after each injection as described in the Procedure Manual. Due to the injection some subjects may experience post procedural pain requiring an overnight stay. Post procedural pain requiring a single overnight stay in hospital will not be reported as an SAE (unless other SAE criteria are also fulfilled). Post procedural pain will be captured as an adverse event and summarised separately in the CSR. Subjects will be advised to undertake only modest activity levels that do not provoke pain between injections and for the first 14 days following the last injection, and only engage in activity within pain limits thereafter.

Part B	**Dose (PP353/Placebo)**	**Number of Subjects**
Arm 1	Two injections of up to 3 mL of 50 mg/mL of linezolid suspension of PP353	20
Arm 2	Two placebo injections	20

OBJECTIVES:

The specific objectives are:

Objectives	**Part A**	**Part B**
Primary	- To evaluate the safety and tolerability of PP353 when administered by intradiscal injection - To characterise the PK profile of a single dose of PP353 in plasma to enable a choice of dosing regimen to be used in Part B to give approximately 8 days of intradiscal exposure to PP353 as inferred from plasma exposure	- To evaluate the safety and tolerability of PP353 when administered by intradiscal injection - To evaluate the efficacy of PP353 as measured by change from baseline in LBP NRS score at 12 months
Secondary		- To characterise the PK profile of PP353 in plasma for the dosing regimen selected in Part A to give approximately 8 days of intradiscal exposure to PP353 in the

		active arm as inferred from plasma exposure - To evaluate the efficacy of PP353 as measured by change from baseline in LBP NRS score at 3, 6 and 9 months - To assess the efficacy of PP353 as measured by improvement in disability due to CLBP
Exploratory Part A and Part B	- To evaluate the relationships between drug exposure, efficacy and subject reported outcomes - To explore imaging biomarkers related to quantitative assessment of inflammation and oedema in the spine - To characterise the PK profile of selected excipients from dose(s) of PP353 in plasma (analysis will depend on the stability of the PK samples already collected and the consent of the subjects if consent has not already been obtained for such analysis) - To investigate if systemic (blood) cytokine levels measured at baseline is related to response to treatment.	
Exploratory Part A	- To evaluate the efficacy of PP353 as measured by change from baseline in LBP NRS score at 3, 6, 9 & 12 months - To assess the efficacy of PP353 as measured by improvement in disability due to CLBP	

Exploratory Part B		- To evaluate the efficacy of PP353 as measured by change from baseline in average LBP daily score over a 7-day period at 3, 6, 9 & 12 months

ENDPOINTS:

PRIMARY ENDPOINTS

Primary endpoints for Part A and Part B:

- Safety variables

Incidence of adverse events, throughout the study.

Primary endpoint for Part A only:

- Pharmacokinetic parameters of PP353 following injection

Primary endpoint for Part B only:

- Efficacy variables

Primary efficacy variable:

- Change from baseline in LBP NRS score at 12 months.

  The LBP NRS score throughout this protocol is defined as the average of the score of the three questions:

  1. Low back pain intensity now
  2. Worst low back pain intensity in the last 14 days
  3. Average low back pain intensity over the last 14 days

  Each question will be assessed by the subject on an 11-point scale with 0 = "no pain" and 10 = "the worst possible pain you can imagine." The average of the three scores will be used to summarise the subject's low back pain.

SECONDARY ENDPOINTS

Secondary endpoints for Part B:

- Pharmacokinetic parameters of PP353 following injection
- Secondary efficacy variables:

- Change from baseline of LBP NRS score at 3, 6 and 9 months
- Change from baseline in RMDQ-23 score at 3, 6, 9 and 12 months
- Clinically relevant improvement at 3, 6, 9 and 12 months, defined as 30% reduction from baseline in the RMDQ-23 score
- Change from baseline in Oswestry Disability Index (ODI) at 3, 6 and 12 months

EXPLORATORY ENDPOINTS

Exploratory endpoints for Part A and Part B:

Exploratory safety variables:

- safety laboratory tests, vital signs, electrocardiograms (ECGs) throughout the duration of the study

Exploratory efficacy variables:

The following subject reported outcomes and DCE-MRI and/or MRI assessments will be evaluated at all timepoints assessed:

- Change from baseline in hours with low back pain during the last 4 weeks
- Subject global perceived effect
- Change from baseline of leg pain NRS score

  Leg pain NRS score will be determined using the same subject-reported scoring system used for LBP NRS.
- Change from baseline in use of analgesic medication
- Change from baseline in RMDQ-24 score

- Changes from baseline in the volume and severity of vertebral body endplate oedema and inflammation, as captured by DCE-MRI and/or MRI
- Exploratory Pharmacokinetics: selected excipients from dose(s) of PP353
- Baseline blood cytokine levels and relationship with response to treatment.

Exploratory endpoints for Part A only:

- Change from baseline in LBP NRS score at 3, 6, 9 & 12 months
- Change from baseline in RMDQ-23 score at 3, 6, 9 and 12 months
- Clinically relevant improvement at 3, 6, 9 and 12 months, defined as 30% improvement in the RMDQ-23 score
- Change from baseline in ODI at 3, 6 and 12 months

Exploratory endpoints for Part B only:

- Change from baseline of average LBP intensity NRS daily score over a 7-day period at 3, 6, 9 & 12 months

  Each question will be assessed by the subject on an 11-point scale with 0 = "no pain" and 10 = "the worst possible pain you can imagine." For the 7 days prior to the scheduled visits, subjects will record the number that best describes their average low back pain over the past 24 hours.

Number of subjects (planned):

The number of subjects to be recruited in Part A is up to six evaluable subjects. As an adequate PK profile is required for evaluation and to inform the selection of the number of injections to be administered in Part B, drop-outs in Part A will be replaced if adequate PK profile is not obtained.

In Part B, 40 subjects will be enrolled. Subjects dropping out will not be replaced.

INCLUSION/EXCLUSION CRITERIA

Inclusion criteria

To be eligible to participate in this study, all the following criteria must be met by all subjects at the timepoints indicated:

	Inclusion Criteria	Screening	Day 1 pre-randomisation
1.	Can understand and sign the written informed consent form (ICF).	X	
2.	Considered reliable and capable of adhering to the protocol.	X	X
3.	Aged between 18 and 70 years, inclusive.	X	

4.	CLBP in the area L1 to S1 associated with vertebral body endplate bone oedema (Modic 1) or vertebral body endplate bone oedema and fat (Modic 1 and 2) at a single lumbar level. *Modic 1 or Modic 1 and 2 are allowed at non-lumbar levels e.g., cervical. Modic 2 is allowed at levels more than 2 vertebrae away from the target with the limitations described in exclusion 3.1. Modic 3 (Subchondral bone sclerosis) is permitted at any level.*	X[1]	X[2]
5.	Current episode of CLBP has lasted for ≥ 6 months at the time of randomisation.	X	X
6.	Average LBP NRS score (from 14-day recall question, Appendix B Subject Questionnaire) at screening and at Day 1 pre-randomisation of ≥ 4 on chronic pain medication and ≥ 6 if not on chronic pain medication; it should be higher than the leg pain NRS score. *Chronic pain medication is defined as taking analgesia on at least 4 days out of 7 in the week prior to screening and the week prior to randomisation. Exceptions to this criterion are permissible following discussion between the Sponsor study physician and investigator.*	X	X
7.	RMDQ-23 score ≥ 9 at screening and at Day 1 pre-randomisation.	X	X
8.	Central review of imaging confirms the disc changes are suitable for the study and there are no other significant spinal pathologies that could account for the pain symptoms.		X
9.	Bodyweight of ≥ 50 kg and ≤ 120 kg. Subject is able to undergo required protocol procedures and in the opinion of the investigator, the target lumbar disc is likely to be injected successfully. *Exceptions to this criterion are permissible following discussion between the Sponsor study physician and investigator.*	X	X
10.	Failure of standard of care therapies used by their treating physician	X	
11.	Agrees to use highly effective contraception*:		
a.	Female subjects of child-bearing potential must agree to use dual methods of contraception (including one highly effective and one effective method of contraception*) from the start (signing consent) until 1 month after the end of the study and have a negative serum pregnancy test at screening.	X	
b.	Male subjects must use an acceptable effective* barrier method of contraception if sexually active with a female of child-bearing potential from the start of the study until 100 days after the last injection.	X	

c.	Male participants agree not to donate sperm until 100 days after the last injection.	X	

**Highly effective and acceptable effective methods of contraception are described in Section 5.2.10 of the protocol.*
[1] Based on historical or suitability MRI, if available
[2] Eligibility MRI

Exclusion Criteria

Subjects are not permitted to enrol in the study if any of the following criteria are met:

		Screening	Day 1 Pre-randomisation
1.	No longer applicable.	NA	NA
2.	The target lumbar disc has lost more than half its original anticipated height at the centre or it is < 5 mm in height over the central 15 mm portion. *Exceptions to this criterion are permissible in cases where the investigator believes the target disc is accessible to injection and following discussion between the Sponsor study physician and investigator.*	X[1]	X[2]
3.	No longer applicable.	NA	NA
3.1	Any vertebra with Modic 2 only lesions which: a) in the opinion of the investigator, after deep palpation of the vertebral spine, is contributing to the low back pain and/or b) are present within 2 vertebrae from the target lumbar disc. *For clarification: If the target disc is L5/S1, Modic 2 in the L3 vertebra would be permissible provided the investigator does not think that lesion is contributing to the back pain.*		
4.	Gross facet joint degeneration or cases where the investigator believes the primary pain generator to be the facet joints.	X[1]	X[2]
5.	In the investigator's clinical judgement, there is a clear alternative cause for back pain, e.g. inflammatory joint disorders.	X	
6.	Therapeutic interventional back procedure in the 6 months (26 weeks) prior to screening, with the exception of corticosteroid injections which are excluded in the 3 months (13 weeks) prior to screening. *Diagnostic procedures, or procedures which only provide transient pain relief e.g. epidural injection, are permitted but the Investigator should allow sufficient time*	X	

	(at least 4 weeks) after the diagnostic procedure for the subject's pain to have settled back to their normal levels before screening.		
7.	History of alcohol abuse or drugs of abuse within the past two years.	X	
8.	No longer applicable.	NA	NA
9.	No longer applicable.	NA	NA
9.1	Taking any prohibited medications. *See section 4.4.2*	X[3]	X[3]
10.	No longer applicable.	NA	NA
11.	Unable to temporarily stop using anticoagulant or antiplatelet treatment around the time of the injection. The use of low dose aspirin (up to and including 100 mg per day) is permitted. *See section 4.4.2*		X[3]
12.	Poorly controlled diabetes mellitus type 1 and type 2. *A poorly controlled diabetic defined as having an HbA1c level greater than 64 mmol/mol (8%) in the preceding 6 months will be excluded from the study.*	X	
13.	Poorly controlled hypertension.	X	
14.	Female subject who is pregnant or plans to become pregnant during the study, or is breastfeeding.	X	X
15.	Received any investigational drug or experimental procedure to treat their low back pain within 180 days prior to screening.	X	
16.	Previously been treated with antimicrobial agents for their low back pain or previously received any antimicrobial intradiscal injection.	X	
17.	Active infection (e.g., sepsis, pneumonia, abscess) in addition to the suspected chronic discal infection. Or Has taken a course of antibiotic treatment for > 14 days within 6 months prior to study drug administration. *When in doubt, the investigator should confer with the Sponsor study physician and in certain cases the subject may be included*.	X	X

18.	Required to take or likely to take prophylactic antibiotic therapy for whatever reason over the duration of the study. The use of prophylactic antibiotic administration in the peri-surgery period, other than linezolid, is permitted as per institution policy.	X	X
19.	No longer applicable.	NA	NA
20.	No longer applicable.	NA	NA
21.	Platelet count of < 100 x 10^9/L	X[4]	
22.	White cell count < 3.0 x 10^9/L (3000/mm^2) or absolute neutrophil count (ANC) < 2.0 x10^9/L (2000/mm^2)	X[4]	
23.	Reduced kidney function, defined as an eGFR of < 45 mL per min per 1.73m^2, calculated by the Modified Diet in Renal Disease (MDRD) study equation. *Subjects with eGFR of 45-60 mL per min per 1.73m2 can be included in the study but* ***must not*** *receive gadolinium during any study MRIs.*	X[4]	
24.	Renal or liver impairment, defined as:		
a.	For women, serum creatinine level ≥ 125 µmol/L; for men, ≥ 135 µmol/L, or	X[4]	
b.	Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≥ 1.5 x upper limit of normal (ULN), or alkaline phosphatase (ALP) and/or bilirubin > 1.5 x ULN *Subjects with a diagnosis of Gilberts disease with bilirubin up to 3 x ULN may be included after discussion and agreement with the Sponsor study physician.*	X[4]	
25.	HIV positive, history of or current hepatitis, or carriers of HBsAg and/or anti HCV.	X	
26.	Active neoplastic disease or history of neoplastic disease within 5 years of screening (except for basal or squamous cell carcinoma of the skin or carcinoma *in situ* that has been definitively treated with standard of care).	X	
27.	Progressive visual loss or macular degeneration. Closed angle glaucoma or progressive cataract occlusion are permitted.	X	
28.	Major surgery within the previous 12 weeks prior to screening. Major surgery is defined as surgery requiring general anaesthesia, or a surgery that lasts longer than 30 minutes, is not performed laparoscopically or is not for a trivial dermal procedure (e.g., wart removal or excision of benign lesion/tumour).	X	
29.	Impaired cardiac function or clinically significant cardiac diseases, including any of the following:		
a.	Unstable angina or acute myocardial infarction ≤ 12 weeks prior to screening;	X	

b.	Clinically significant heart disease (e.g. symptomatic congestive heart failure [e.g. more severe than New York Heart Association [NYHA] Class 2]; uncontrolled arrhythmia).	X	
30.	Serious psychiatric or medical conditions including but not limited to: subjects with phaeochromocytoma; carcinoid; thyrotoxicosis; bipolar depression; schizoaffective disorder; acute confusional states that, in the opinion of the investigator, could interfere with treatment, compliance, or the ability to give consent.	X	
31.	History of hypersensitivity or allergies to linezolid, poloxamer 407 gel, iohexol or iodine; or documented intolerance to required medication for the procedure e.g. local anaesthestics (lignocaine, bupivicaine and marcaine) or systemic anaesthetics e.g propofol.	X	
32.	Acute clinically significant illness within 30 days prior to study drug administration.		X
33.	Contraindication to MRI examination including, but not limited to, intracranial metal clips, heart pacemakers, insulin pumps, implanted hearing aids, neurostimulators, metal hip replacements, profound claustrophobia, or inability to lie in the MRI machine in an appropriate position to obtain quality images. *Subjects with a history of allergic reactions to gadolinium may be enrolled in the study but **must not** receive gadolinium during any study MRIs.*	X	
34.	Subjects who do not have suitable veins for multiple venepunctures/cannulation as assessed by the investigator at screening.	X	
35.	Subjects with significant medical comorbidities that, in the opinion of the investigator, should not participate in the trial.	X	X
36.	Participation in any other clinical research study within 90 days prior to screening and for the duration of Persica 002.	X	

[1] *Based on historical or suitability MRI, if available*

[2] *Eligibility MRI*

[3] *Review concomitant medications at screening for contraindicated and prohibited medications*

[4] *If local laboratory tests are performed pre-surgery as routine practice, any clinically significant abnormalities detected are to be reported as an adverse event. If any of the local lab results are exclusionary, the investigator must discuss with the Sponsor study physician prior to dosing*

Investigational product, dosage and mode of administration

PP353 has been formulated to allow intervertebral disc injection of the active ingredient linezolid. The diluent contains poloxamer 407 and a contrast agent to enable delivery and to allow image-guided dosing until the intervertebral space is full and the PP353 starts to leak out from the intervertebral space (extravasation).

The drug product will undergo suspension using aseptic technique, must be kept at 2–8ºC until administration and must be used within 3 hours of suspension. Detailed instructions are provided in the Persica Study 002 Pharmacy Manual.

Dosage = 3 mL of 50 mg/mL linezolid suspension.

PP353 will be injected directly into the affected intervertebral disc, as shown by MRI (vertebral body endplate bone oedema [Modic 1] or vertebral body endplate bone oedema and fat [Modic 1 and 2] at a single lumbar level).

STATISTICAL METHODS:

General statistical methods:

The results of the two parts of the study will be analysed separately.

Sample size justification:

Part A: No formal power calculation was performed for Part 1. The sample size of three subjects with the possibility of an additional three is considered sufficient for the PK analyses to determine the choice of regimen for Part B. The decision to use an additional three subjects will be evaluated on:

- The variability of the PK of the first three subjects and whether the data provide a reliable and consistent PK profile

Part B: Power calculations were based on the results of the large CLBP randomised control trial (Albert *et al*, 2013b). That study's database was used to select the subset of subjects with baseline RMDQ score of ≥ 9 and baseline LBP NRS score of ≥ 4, and higher than the leg pain NRS score to fit the inclusion criteria. The subjects receiving antibiotics had a mean reduction from baseline in the LBP NRS score at 1 year of 3.28 units and placebo subjects had a reduction of 0.61 units. A conservative reduction of 1.2 units for the placebo group, as estimated from the AIM study (Bråten et al., 2019) was used rather than a reduction of 0.61 units in the final sample size calculation.

sample size of 20 subjects per arm (taking into account 20% for withdrawals) is considered sufficient to detect a difference in CLBP reduction, compared with baseline, between the active and placebo groups of at least 2.08 units, assuming a common standard deviation of 2.3. A one-sided, two-group comparison with 5% type I error using a t-test provides power greater than 80%.

Evaluation for safety:

All safety variables will be analysed using descriptive methods. Concomitant medications and procedures will be listed.

Pharmacokinetic analyses:

PK parameters will be determined, enabling a choice of dosing regimen that is estimated to give approximately 8 days' exposure within the disc.

Evaluation for efficacy:

Part A: The exploratory efficacy results will be listed and summarised.

All other efficacy variables will be listed, summarised, and analysed (where appropriate), using statistical testing.

Part B: The primary efficacy endpoint will be analysed using a mixed model for repeated measures to test the difference in the two groups for the LBP NRS score change from baseline at 12 months.

The primary efficacy endpoint may also be analysed using Bayesian analysis with an informative prior for the placebo group, if appropriate.

The secondary efficacy variables will be listed, summarised, and analysed (where appropriate) using statistical testing.

All other efficacy variables will be listed, summarised, and analysed (where appropriate), using statistical testing.

Interim analyses:

Interim analysis – Part A:

The SRC will conduct a formal review of the safety, tolerability and PK data after the first three subjects in Part A have completed PK sampling. Data will be reviewed to identify the dosing regimen and timings of data collection for Part B of the study. If data for the first three subjects are evaluated and are not adequate to reach a decision on the choice of regimen for Part B, an additional three subjects will be enrolled in Part A and the review repeated once the second cohort in Part A have completed the PK sampling and analysis.

In addition to the review of the safety and PK data for Part B dosing regimen decision, the SRC will also review the utility of the contrast enhanced MRI of available images from all the subjects in Part A and available Part B screening MRIs to make recommendations on the scientific value of inclusion of the 6 month DCE-MRI scan in Part B of the study.

Early read-out - Part B:

In Part B, there may be up to TWO Sponsor-unblinded early read-outs, both following recruitment of all subjects. It is likely that the first will be conducted after all subjects have completed the Month 6 visit and the second after all subjects have completed the Month 9 visit. This will not alter the conduct of the Persica 002 study. Analyses will be conducted to enable the Sponsor to plan for future studies and for confidential investor discussions important to the future of Persica Pharmaceuticals Ltd. Details of the early read-out analyses may be provided in a separate statistical analysis plan (SAP).

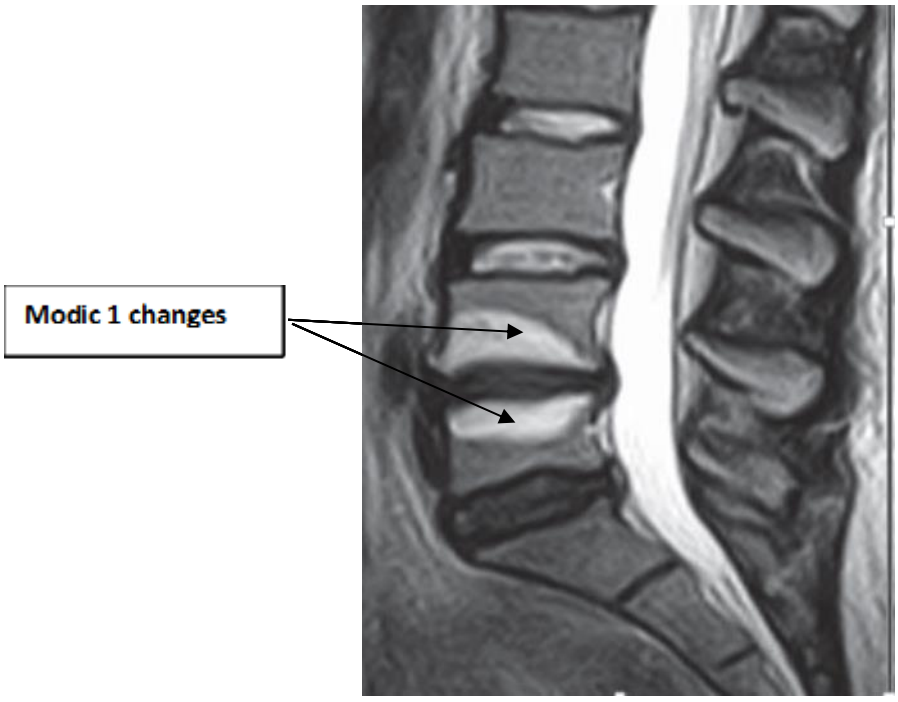
1. Introduction

1.1 Chronic Low Back Pain (CLBP) and Modic Changes

Chronic low back pain (CLBP) is the leading global cause of disability and a major health economic issue for all developed countries (Vos *et al*., 2013). CLBP treatments are largely ineffective, including conservative therapies (Airaksinen *et al*., 2006), medication (Qaseem *et al*., 2017) spinal injections (Stall *et al*., 2008) and spinal fusions (Mirza *et al*., 2007). Many of the existing treatment options for CLBP are ineffective and the use of strong analgesic medication is common.

There is widespread agreement that a precise pathoanatomical diagnosis can only be made in 15%–20% of subjects (Airaksinen *et al*., 2006). However, there is a compelling body of evidence that supports the hypothesis that disc herniations may initiate a series of events that in turn lead to bacteriologically-induced inflammation, degeneration and oedema. Vertebral body endplate oedema can be visualised by MRI imaging and is termed as Modic 1 changes (Modic *et al*, 1988). In addition, vertebral body endplate fissures where fatty deposits and fibrovascular tissue are found in and around the damaged vertebral endplates are commonly referred to as Modic 2 changes and can be seen in association with Modic 1 (Albert *et al*., 2008b; Modic *et al*., 1988).

Figure 1: T2 Weighted MRI scan showing Modic changes type 1



Recognition of Modic 1 and Modic 2 changes shows very high interrater kappa values (Wang *et al*., 2011), and they can be semi-quantitatively assessed (Jones *et al*., 2005; Jensen *et al*., 2007). Modic 1 and/or Modic 2 are observed in approximately 6% of the general population, and at variable rates of between 20% and 60%, median value 43%, in subjects with low back pain (Jensen *et al*., 2008). In a large MRI study of 40-year-old individuals from the general population, the strongest associations with low back pain were Modic 1 and Modic 2 changes and anterolisthesis (Kjaer *et al*., 2005).
No papers properly focus on the microbiology of discs from the Persica 002 study target subject population: Modic 1 or Modic 1 and 2, CLBP > 6 months, back pain > leg pain. This group was selected as they are the subset that responded the most to oral antibiotics in the Albert *et al*., 2013b study. It is considered the most efficient subject subset to test the efficacy of PP353.

The presence of bacteria in herniated disc samples and their contribution to disease has been controversial, with some claiming that contamination from the surgical sampling procedure is the source of bacteria, others that bacteria are present throughout tissues, but their number in herniated disc samples is greater than in other tissues and they may contribute to disease, and many that conclude that bacteria in discs, and particularly *Propionibacterium acnes*, is associated with, and probably causal of Modic disc degeneration (Urquhart *et al*., 2015).

The study targeting subjects most alike the Persica 002 subject population, analysed 385 disc samples from 313 subjects with CLBP for > 1 year (Rigal *et al*., 2016). But the study was technically flawed as they failed to provide adequate anaerobic growth conditions (Czaplewski, 2016) and it is hardly surprising that they failed to culture anaerobic bacteria. Their conclusion that poor aseptic technique and tissue contamination during surgical procedures are responsible for bacterial identification in disc samples was flawed. Two other studies, Ben-Galim *et al*., (2006) and Alamin *et al*., (2017) also reported low levels of bacterial infection of herniated disc samples and concluded that contamination is a likely source.

It is commonplace to provide prophylactic antibiotic coverage during surgery. Many provide prophylaxis during anaesthesia, others post-surgery, e.g., 1–2 g of a cephalosporin. Clinical experts comment that they don’t think a broad-spectrum cephalosporin will inhibit recovery of bacteria from disc samples; but prophylactic therapy may well reduce bacterial burden and

make bacterial recovery harder. It is likely that prophylactic antibiotic use may cause an underestimation of infected disc number.

In 15 studies, spanning 2001 to 2018, evaluating 1516 discs, 598 were infected with a range from 0% to 52.8% and a mean of 39.4%. (Stirling *et al*., 2001; Fritzell *et al*., 2004; Ben-Galim *et al*., 2006; Carricajo *et al*., 2007; Agarwal *et al*., 2011; Arndt *et al*., 2012; Albert *et al*., 2013a; Zhou *et al*., 2015; Capoor *et al.*, 2016; Coscia *et al*., 2016; Aghazadeh *et al*., 2017; Capoor *et al*., 2017; Yuan *et al*., 2017b; Tang *et al.*, 2018). Four studies describe assessment of Modic discs in enough detail to analyse. 180 Modic discs were described and 86 were infected with a range of 0% to 60% and a mean of 47.8%. The most prevalent bacterial species identified was *P. acnes*.

The Alamin *et al*. study (2017) did not identify any infected subject samples. This study only used polymerase chain reaction (PCR) and did not attempt culture to detect bacterial infection. Others have found that PCR and culture do not necessarily correlate because of the difficulty in disrupting disc tissue and disruption of bacterial aggregates (Ohrt-Nissen *et al.,* 2018). The Alamin group used central laboratory services to provide PCR technology rather than developing and optimising assay methodology specific for disc tissue.

Albert *et al*., (2013a) followed subjects with infected herniated discs and found that 80% of subjects went on to develop Modic changes in vertebrae adjacent to the herniation vs 44% of subjects with sterile herniated material. This observation suggests that bacterial infection may be associated with development of Modic change.

The microbiological methodology employed largely provides a qualitative assessment of bacterial infection; a binary yes or no. Analysis of species diversity is also qualitative. A small number of studies have started to develop semi-quantitative methods but no deep sequencing or validated quantitative assessment of herniated disc samples has been published. In two large studies of 290 and 368 discs, bacteria were identified in 130 and 162 discs, *P. acnes* in 115 and 119 of these and *P. acnes* burden was estimated to be between 120 to 9000 CFU/mL with a median of 400 CFU/mL of disc in the first, and between 12 to 20,952 CFU/g disc, with a median 350 CFU/g in the second study. (Capoor *et al*., 2016; Capoor *et al*., 2017). Adequate disc tissue homogenisation was considered key to sample preparation. The authors concluded that in many discs, *P. acnes* were present in too high a number to be explained by contamination alone.

Histology has confirmed the presence of bacterial aggregates in disc tissue which is unlikely to be due to contamination (Yuan *et al*., 2017a; Ohrt-Nissen *et al*., 2018). Proteomic analysis of discs detected bacterial proteins at concentrations which could not feasibly be due to contamination during harvesting (Rajasekaran *et al*., 2017).

As appreciation of bacterial presence in internal tissues and blood develops, it may be that bacteria and especially *P. acnes* is present at low burden in many tissues but in some cases, like degenerate discs and herniated material, they can grow and increase in number. It will be difficult to unambiguously assign cultured bacteria as infection or contamination and, in many cases, both may be present. When disc samples have been tested from subjects with spinal disorders other than herniation, Modic or diagnosed infective discitis, disc samples tend to be sterile (Stirling *et al*., 2001: Chen *et al*., 2018).

Furthermore, in alignment with Koch's postulates of microbial pathogenicity, transfer of human-derived *P. acnes* from subjects with Modic 1 changes to rabbits and rat tail intervertebral discs has been shown to lead to degeneration and vertebral Modic 1-like changes (Chen *et al*., 2016: Dudli *et al*., 2016).
In cells extracted from intervertebral discs and co-cultured with *P. acnes* derived from a subject with Modic 1 changes, multiple cytokines are upregulated including IL-1, IL-6, IL-8 and CCL23, demonstrating in the presence of these microbes the discs could generate an immunological response to drive vertebral Modic 1 change (Dudli *et al*., 2018).

A recent publication observed that intervertebral disc samples adjacent to Modic 1 changes from patients undergoing lumbar spinal fusion had either 'high' or 'low' genome copy numbers (GCNs) of *P.acnes*. Modic 1 changes in patients with "high" *P. acnes* GCNs had normal cytokine blood plasma levels whereas those with "low" GCNs had high cytokine levels (Heggli *et al.*, 2024). If replicated this may suggest that subjects with CLBP and raised cytokine levels are less likely to have an infectious pathology than those with a low or normal systemic cytokine levels.
In summary, there is compelling clinical and scientific evidence that disc infection with anaerobic bacteria is associated with and may give rise to a significant proportion of subjects suffering from CLBP and that with routine MRI, Modic 1 and Modic 2 changes may be used as a marker to identify a population enriched for these subjects.

In humans with CLBP associated with Modic 1 changes, the effects of treatment with oral antibiotics (amoxicillin-clavulanic acid) have been reported in one prospective uncontrolled

trial (Albert *et al*. 2008a), one large double-blind randomised trial (Albert *et al*., 2013b), one smaller randomised trial (Al-Falahi *et al*, 2014) and one large open label trial including ≥ 1000 subjects (Albert, 2017), as well as an audit from a university hospital (Manniche *et al*., 2016). All these studies have demonstrated significant benefit of treatment with amoxicillin-clavulanic acid for 90 or 100 days.

In the Albert (2013b) randomised controlled trial (n=162), there was a statistically significant improvement in the treated group compared with the placebo group on primary and secondary outcome measures at 1-year follow-up. The measures included leg and lumbar pain, and a disease-specific questionnaire with a dynamic range of 24 (RMDQ-23). The 12-month MRI scans demonstrated a decrease in volume of the Modic 1 oedema compared with the placebo group. Also, a trend towards a dose-response relationship was observed with double-dose antibiotics, suggesting that antibiotic doses may determine effective penetration of the disc and treatment of the infection.

The open label study and the smaller randomised trial (undertaken by a separate group) both showed positive results of similar magnitude in this subject population. (Al-Falahi *et al*, 2014; Albert *et al*., 2008a).

An open label study of over 1000 subjects with CLBP and Modic 1 and /or Modic 2 changes on MRI and who were treated with 100 days of Bioclavid™ / Augmentin™ (amoxicillin/clavulanate potassium 1 g/250 mg tds) demonstrated sustained improvement beyond 1 year (n=602) and beyond 2 years (n=270), mirroring in a real world setting the controlled trial data (Albert *et al*., 2013b). The remaining subjects had not reached the follow-up times at the time of publication.

The use of high dose oral antibiotics for 90–100 days is challenging as they are not well tolerated, with 48% of subjects receiving oral antibiotics in the large randomised controlled trial (RCT) suffering “middle to considerable” side-effects, mostly GI, compared with 17% in placebo groups and 4.4% of subjects receiving antibiotic treatment stopping due to adverse effects of the therapy (Albert *et al.*, 2013b).

1.2 Advanced MRI Sequences and Quantitative Assessment of Inflammation

Gadolinium (Gd)-enhanced MRI will be used to visualise increased contrast uptake in inflamed tissues due to hyper vascularisation and altered biochemical characteristics. It has been used to demonstrate degenerative-inflammatory changes of the facet joints and posterior elements of the lumbar spine (Lehman *et al*., 2013). Perfusion MRI, also known as contrast enhanced (CE)-MRI uses a Gd-based contrast agent, which is injected intravenously during

image acquisition. The MRI signal depends on the T1 relaxation time (MRI scanner parameter) of the contrast agent in the blood and therefore, areas of high vascularity and perfusion become hyperintense, i.e. bright in the images.

Contrast-enhanced MRI can be acquired at a single time point or dynamically over time. Dynamic contrast enhanced (DCE)-MRI captures the contrast agent distribution over time and provides sensitive and specific information about the local perfusion. Rapid and repeated T1-weighted images are acquired to visualise the perfusion of contrast medium over time. Detailed contrast accumulation is recorded and allows for a pixel-based contrast enhancement curve analysis. DCE-MRI is a sensitive method to provide detailed characterisation of inflammation in degenerative joints (Boesen *et al*., 2018a and 2018b).

The DCE-MRI data are quantified using image post-processing techniques to examine the microvasculature through quantitative assessment of microvascular permeability. This is of particular relevance in clinical evaluation and clinical research as the perfusion changes provide valuable insights into the therapy efficacy.

1.3 Background on PP353

1.3.1 Non-clinical Background

PP353 has been specifically formulated to allow the delivery of a linezolid depot to the intervertebral disc and intervertebral space with a duration of efficacious local exposure of approximately 8 days by the administration of one to three intradiscal injections. Linezolid is an antibiotic with a large safety database through its clinical use with both intravenous and oral formulations. The frequency of linezolid-resistant Gram-positive bacteria is very low which is important as bacterial sampling is not possible in this condition.

PP353 consists of its active ingredient linezolid, a diluent containing poloxamer 407 to aid drug delivery/localisation, and iohexol, an image contrast agent to enable/confirm accurate injection into the intervertebral disc and detect evidence of extravasation from the disc and intervertebral space. All three of these agents are well precedented in humans and individually approved by the EMA and/or FDA, in some cases in combination with other agents. To prevent the formulation leaking out of degenerate discs, poloxamer 407 was selected as it undergoes a temperature-dependent increase in viscosity on warming going from a liquid at room temperature to a stiff gel at 36°C. The temperature at which the

viscosity increases is known as the sol gel transition. The sol gel of PP353 is within the range of 26°C to 36°C. This gel should retain the insoluble linezolid at the site of injection.

Preclinical studies have demonstrated that PP353 has anti-microbial activity when injected into a large animal model of disc infection. In addition, a preclinical tolerability study has been conducted which demonstrated local tolerability of PP353 at the clinically relevant concentration of 50 mg/mL (5 mg/disc) of linezolid. Although there were some gross changes (discolouration) observed in these studies, these were considered to be related to the procedure rather than PP353 (for further discussion on the local tolerability study, please see the IB Section 6.1).

1.3.2 Non-clinical Studies

Linezolid is approved for oral and i.v. clinical use for the treatment of community acquired pneumonia and nosocomial pneumonia when known or suspected to be caused by susceptible Gram-positive bacteria, and treatment of complicated skin and soft tissue infections only when caused by susceptible Gram-positive bacteria, at a dose of 600 mg twice daily for a maximum duration of 28 days. Therefore, the safety and efficacy in humans for the use of linezolid in these indications is well-established. With regards to the standard battery of non-clinical safety and toxicology studies, reference is made to the approved summary of product characteristics (SmPC) and literature.

Intradiscal administration is a form of parenteral administration, delivering the antibiotic directly into the disc. This minimises systemic exposure and potentially reduces systemic side effects. Linezolid is highly bioavailable with similar exposure achieved by i.v. and oral dosing. A standard 10–28-day course of treatment exposure to linezolid would be between 12 g and 33.6 g throughout the therapy. Persica proposes to provide approximately 8 days of intradiscal linezolid exposure using one, two or three injections. The number and timing of the injections will be informed by human PK.

As linezolid diffuses from the treated disc it will distribute throughout the body and be eliminated. The planned dose of linezolid and the relatively slow solubilisation and diffusion of linezolid from the disc are expected to result in a significantly reduced maximum and overall level of systemic exposure to linezolid than achieved through i.v. administration of the approved dose.

As linezolid is well tolerated when dosed to humans for 10–28 days in accordance with the approved posology, and intradiscal administration represents a significant reduction in systemic exposure, additional systemic toxicology testing for PP353 is not considered

necessary. This approach is consistent with the CHMP guideline on non-clinical local tolerance testing of medicinal products (EMA/CHMP/SWP/2145/2000, 22 October 2015). There is also precedent for the parenteral administration of iohexol (Omnipaque™, UK PL 00637/0036) and poloxamer (LeGoo®, Sanofi-Aventis/ Pluromed; Shalhoub *et al*., 2013; Decrouy-Duruz *et al*., 2013; Mani *et al*., 2013). There is significant precedent for the intradiscal injection of iohexol during provocative discography (Pobiel *et al.* 2006).
Persica has conducted the *in vivo* non-clinical efficacy studies with the research formulation, intradiscal pharmacokinetic studies with the development formulation and good laboratory practice (GLP) local tolerance study with the good manufacturing practice (GMP) formulation.
All pivotal *in vivo* non-clinical studies (i.e. those studies identified in International Council for Harmonisation (ICH) guidelines as needing to be conducted in accordance with GLP were conducted in the UK, a country that is a signatory of the OECD Mutual Acceptance of Data (MAD) system in accordance with the OECD Test Guidelines and Principles of Good Laboratory Practice.

Summary of non-clinical study results
All the pharmacodynamics and PK studies were conducted in commercial farm Charollais cross male sheep:

- The pharmacodynamics study (Study CU3 NSPK20-09-16) demonstrated that co-localised dosing of linezolid with the bacteria is not required for efficacy. No health issues were reported during the study and no veterinary intervention was required for any animal. However, during the recovery period post-dosing, 4 of the 11 sheep were recorded as being slow to recover and/or having stiff left hind legs for a period. All sheep were recorded as walking normally by the following day. This had not been recorded in previous studies but was recorded in the first two sheep of the subsequent study (Study CU3-NSPK21-11-16, see Section 4.4.2). The procedure was reviewed, and it was noted that in order to administer both bacteria and antibiotic and to obtain both dorsal-ventral and lateral x-ray images of the injections, the sheep remained anaesthetised in the sternal position for more than two hours. In the following studies, the position of the sheep's hind limbs during dosing was altered and an extra sandbag was placed under the animal's pelvis. The issues with slow recovery or stiff hind limbs was not reported in four subsequent studies using 38 sheep involving 164 intradiscal injections into 116 discs. The conclusion was that the cause of the observations was the time the sheep were in the sternal position and the placement of the hind limbs during the intradiscal dosing.

Changing this position and additional support with sandbags has ensured these observations are no longer seen.

- The non-GLP intradiscal PK and preliminary local tolerance study (Study CU3 NSPK21-11-16) study provided intradiscal PK data on the elimination of linezolid after intradiscal administration of a suspension of micronised linezolid in diluent containing poloxamer 407 and iohexol. A high and low concentration of poloxamer 407 were tested. The rates of elimination for the two formulations were similar, indicating that the diluent containing poloxamer 407 & iohexol does not alter diffusion/elimination from the disc. The formulation was well tolerated in each of the four discs of a single animal at 7 days post-treatment.
- In the non-GLP intradiscal and systemic PK study (CU3 NSPK25-11-17), linezolid concentrations in disc and plasma samples were estimated using a qualified LC-MS/MS method. Four discs in each sheep were injected with 0.1 mL of a development formulation of PP353, linezolid suspension (50 mg/mL) in poloxamer 407/iohexol formulation with a sol gel transition at 32°C. Pharmacokinetic analysis of the samples showed variability between concentrations in the dosed discs, within time points, was moderate to high. The apparent linezolid half-life ($T_{1/2}$) in sheep discs is 5.54 hours. Plasma linezolid concentration vs time profiles were consistent with the extravascular dose route whereby a post-dose elimination from discs and an absorption phase into tissues up to 8 hours after administration was evident followed by a mono-phasic decline in concentrations up to 48 hours. While each disc is considered independent, plasma linezolid concentrations arise from each of the four discs that were dosed (20 mg intradiscal linezolid/animal). Linezolid concentrations in plasma were low compared with disc concentrations with a mean total exposure (AUC(0-t)) in the discs being 18,700-fold higher than in plasma. The apparent $T_{1/2}$ in plasma was 9.35 hours. The GLP local tolerance and systemic PK study (Study CU3-NSPK28-03-18) investigated the local tolerance of PP353 linezolid suspension following intradiscal injection, by assessing if any adverse local effects are present and to observe whether any events were reversible during the recovery period in sheep. Gross changes associated with intradiscal injection, such as a minor increase in dark discolouration, were observed in animals sacrificed at Day 1, Day 14 and Day 28. Microscopic changes observed were haemorrhage, inflammation and muscle fibre degeneration in the soft tissues overlying the target disc compared with un-injected discs. A similar incidence of these changes was seen with intradiscal injection of diluent or PP353, disc needle stick injury and intramuscular injection of the PP353 suggesting that they were related to procedure rather than the test

item itself, and frequency was reduced in animals sacrificed at Day 28 compared with Day 14 and Day 1. Changes to the discs following intradiscal injection observed in animals sacrificed at Day 1, Day 14 and Day 28 were limited to minor increases (of minimal to moderate severity) in matrix disorganisation and cellularity of the nucleus pulposus and eosinophilic material accumulates in the nucleus pulposus and annulus fibrosus when compared to un-injected controls, and frequency was reduced in animals sacrificed at Day 28 compared with Day 14 and Day 1. Similar changes were observed with injection of either diluent or test item and also in needle stick injured discs suggesting they were related to procedure rather than the test item. Systemic exposure to linezolid (based on dose normalised AUC(0-inf)) was comparable between dose routes with a mean relative bioavailability (Frel) of 100%. However, C_{max}/D was 3.0-fold lower and T_{max} was later following intradiscal administration compared with the intramuscular route. No differences in mean $T_{1/2}$ between dose routes were noted. There were no changes associated with PP353 that were thought to be clinically related.

1.3.3 Clinical Safety Background

This is the first study of PP353 in humans. PP353 consists of its active ingredient linezolid, a diluent containing poloxamer 407 to aid drug delivery/localisation, and iohexol, an image contrast agent, to enable/confirm accurate injection into the intervertebral disc and detect evidence of extravasation from the disc and intervertebral space. All three of these agents are precedented in humans and individually approved by the EMA and/or FDA, in some cases in combination with other agents.

Linezolid is approved for oral and intravenous clinical use for the treatment of community acquired pneumonia and nosocomial pneumonia when known or suspected to be caused by susceptible Gram-positive bacteria, and treatment of complicated skin and soft tissue infections only when caused by susceptible Gram-positive bacteria, at a dose of 600 mg twice daily for a maximum duration of 28 days. Therefore, the safety and efficacy in humans for these indications is well-established.

1.3.4 Pharmacokinetics and Product Metabolism in Humans

PP353 has been administered to three subjects in Part A of this clinical trial (Persica 002). All three subjects were dosed with 3 ml of PP353 with no significant extravasation reported. Administration of 3 ml of PP353 resulted in a plasma C_{max} ranging from 1060 ng/mL to 1500 ng/mL ng/mL at 4 to 12 h post administration. The pharmacokinetic data is consistent with all of the linezolid (150 mg) being accounted for in all subjects. Plasma linezolid concentration

falling below 5 ng/mL is considered an indication that intradiscal linezolid has been eliminated from the intervertebral disc space. The time post dosing to reach 5 ng/mL (T_{dur}) was between 89.4 and 110 hours. This is consistent with dosing on Days 1 and 5 of Part B of this Persica 002 study to provide approximately 8 days of intradiscal linezolid exposure.
The linezolid C_{max} observed with intradiscal PP353 is approximately 10% of that observed with standard *iv* or oral administration of linezolid. The Safety Review Committee considered it appropriate to remove restrictions in Part B on concomitant medications contraindicated with *iv* and oral linezolid because of the lower systemic exposure.

1.3.5 Safety and Efficacy

Three subjects have been dosed sequentially in Part A of this study with 3 ml of PP353 by intradiscal injection without significant issues. No serious adverse events were reported. The SRC have agreed it is safe to progress to Part B of the study.
No human efficacy data for PP353 are available to date.

1.3.6 Marketing Experience

There is no marketing experience with PP353.

1.4 Study Rationale

There is compelling clinical and scientific evidence that chronic low-grade disc infection with anaerobic bacteria gives rise to a significant proportion of subjects suffering from CLBP and that using MRI, Modic 1 and Modic 2 changes may be used as a marker to identify a population of these subjects. Clinical studies in subjects with CLBP associated with Modic 1 changes, have demonstrated significant benefit of treatment with amoxicillin-clavulanic acid for 90 or 100 days. The use of high dose oral antibiotics for 90–100 days is challenging as they are not well tolerated, with 48% of subjects receiving oral antibiotics in the Albert *et al.,* (2013b) RCT suffering “middle to considerable” side-effects, mostly GI, compared with 17% in placebo groups, and 4.4% of subjects having to stop the treatment due to adverse effects of the therapy.
PP353 has been specifically formulated to allow the delivery of a linezolid depot to the intervertebral disc and intervertebral space with a duration of efficacious local exposure of approximately 8 by the administration of two intradiscal injections. Linezolid is an antibiotic with a large safety database through its clinical use with both intravenous and oral formulations. The frequency of linezolid-resistant Gram-positive bacteria is very low which is important as bacterial sampling is not possible in this condition.

The primary goal of this Phase 1b Study (Persica 002) is to demonstrate safety and tolerability of PP353 in otherwise healthy subjects with CLBP and to evaluate the efficacy of PP353 to reduce CLBP. Other measures, such as reduction in disability due to CLBP, reduction in analgesic use and reduction in vertebral endplate oedema changes on MRI, will also be assessed.

1.5 Justification of the Starting Dose

Oral antibiotic therapy for 100 days has been shown to be effective in the treatment of CLBP in RCTs but was poorly tolerated (Albert *et. al*., 2013b). PP353 is a novel formulation of linezolid, designed to provide a prolonged high local exposure at the site of infection – the intervertebral disc without associated high systemic exposure.

Linezolid is poorly soluble (2–3 mg/mL) in aqueous solution but forms a homogenous suspension of mostly insoluble powder in a poloxamer 407/iohexol diluent. At concentrations of 50 mg/mL, a homogenous suspension of mostly insoluble powder is formed. At 200 mg/mL, the suspension was not homogenous. A concentration of 50 mg/mL of linezolid was therefore chosen for PP353 as this allows the maximal amount of linezolid (per unit volume) to be reliably injected into the intervertebral disc space when formulated as a suspension. The volume of PP353 to be administered per injection is 3 mL. This volume was chosen based on precedence from previous intradiscal injection studies (Akeda *et al*., 2017; Strube *et al*., 2017; Zhang H *et al*., 2009; Zhang Y *et al*., 2013; Cohen *et al*., 2007; Miller *et al*., 2006; Yin *et al*., 2014, Sainoh *et al*., 2016; Kumar *et al*., 2017; Centeno *et al*., 2017; Zhang *et al*., 2016; Tuakli-Wosornu *et al*., 2016; De Seze *et al*., 2013; Beadreuil *et al*., 2012; Fayad *et al*., 2007; Peng *et al*., 2007). Injections of 3 mL have been well tolerated and allow the maximal dose (150 mg total) to be injected into the intervertebral space. The systemic exposure is anticipated to be approximately 1/8th of that observed with the usual oral daily dose of linezolid and should be well tolerated as many of the side-effects of linezolid are related to the duration of exposure.

It is not proposed to start at a lower dose than 3 mL of 50 mg/mL as would often be the case in a first in human study, as the subjects recruited into the trial have CLBP associated with Modic 1 changes and potentially a chronic infection within the disc space. A lower concentration than 50 mg/mL or a lower volume than 3 mL would result in a much shorter linezolid exposure resulting in a potentially sub-efficacious dose of the antibiotic. The lack of any findings in the preclinical local tolerability study, the low expected systemic exposure of linezolid and the precedented volume of injection support the starting dose of 3 mL of

50 mg/mL linezolid as PP353. There is no plan to increase the volume of injection or the concentration in this study.

The duration of exposure of PP353 is difficult to predict; however, at 50 mg/mL most of the linezolid in PP353 is insoluble and when this is injected into the disc space a localised depot effect is created. Local concentrations of linezolid in solution are predicted to be significantly above the minimal inhibitory concentration (MIC) of linezolid. The duration of local exposure will correlate with the duration of detectable systemic exposure.

In Part A of the Persica 002 study a single intradiscal dose of PP353 was administered to evaluate the plasma PK to measure the duration of release from the depot injection of linezolid. The first three individuals were dosed sequentially, at least 14 days apart. Data from Part A indicates that the regimen in Part B of the study requires two injections to reach a duration of linezolid exposure in the disc of approximately 8 days. The decision to utilise two doses was made by the Safety Review Committee. A sentinel pair (blinded; one subject on active and one subject on placebo) will be dosed followed by the rest of the cohort no sooner than 14 days after the last injection of the sentinel pair.

1.6 Benefit-risk Assessment

This is the first clinical study with PP353, and as such the safety profile has not been established. Although linezolid, poloxamer 407 and iohexol have all been approved for use in humans, they have not been evaluated in combination or when injected into the intervertebral disc. There are three key risks which need to be managed in the first study:

1. The risk of adverse reactions to linezolid due to its systemic exposure. Linezolid has been approved in the UK for oral and intravenous clinical use at a dose of 600 mg twice daily since 2001. The standard recommendation for the use of linezolid is a course of 10-14 days up to a maximum duration of 28 days. The safety profile of linezolid has been established in more than 2,000 adult patients who received the recommended linezolid doses for up to 28 days. The common AEs reported were diarrhoea, headache, nausea, vomiting and constipation which were all higher on linezolid treatment compared with other antibiotics, whereas the rates of rash and dizziness were comparable amongst all drugs studied. Two doses of PP353 will provide 300 mg linezolid vs 12.0 g of linezolid (600 mg b.i.d for 10 days) for a standard course; a 40-fold reduction. Post-dosing PP353 linezolid Cmax is approximately 10% of that observed during standard iv or oral dosing. The low linezolid Cmax and comparatively low dose in PP353 vs standard administration greatly reduces the likelihood of systemic drug-related adverse reactions and drug-drug

interactions (DDIs). The Safety Review Committee has recommended that SSRI and MAOI drug interactions pose a lower risk with PP353 than with standard linezolid dosing and that no medications need be contra-indicated because of DDIs.

2. The second key risk is the local tolerability of the injection into the disc. This risk has been mitigated to a degree by the preclinical local tolerability study in sheep. However, the volume injected into the sheep disc was significantly lower than that planned in this human study due to the constraints of a small disc volume in the healthy sheep. To inject a larger volume would result in a ruptured disc. Within the clinical trial the risk of poor local tolerability is being managed through the design of the study, with the first subjects only receiving a single injection. Each will be followed for at least 2 weeks post injection before the next subject is treated. Only if the single injections are well tolerated in Part A will the multiple injections be used in Part B. If multiple injections are required, then Part B will start with sentinel dosing. The single injection in 3 subjects in Part A of Persica 002 study was well tolerated.
3. The third risk is of injection into the wrong structure. PP353 has been formulated with iohexol to allow contrast guided injection, reducing the risk of injection into adjacent structures. Training on the surgical / injection technique will be provided prior to subjects being enrolled in the study.

An additional risk in the conduct of Persica 002, although not directly related to PP353, is the use of contrast-enhanced media for MRI scans. It is currently planned that there will be three contrast-enhanced MRI scans of the spine using a macrocyclic contrast agent in this study. One at baseline, the second at 6 months post treatment and the final scan at 12 months post treatment. The use of this agent will be within the approved label, excluding subjects with renal impairment. The DCE-MRI scans will be performed on all the Part A and Part B subjects. The Safety Review Committee agreed to include DCE-MRI scans in Part B of the study at 6 months as the information seen in the Part A scans and available Part B screening MRIs suggest sufficient additional information will be gained from their collection.

As a first in human study, due care and attention will be given to evaluation of emerging adverse events, laboratory data, etc, with suitable communication plans to investigators and with the facility to rapidly halt dosing on review of the safety data by a safety monitoring committee. These control features, the recognition that existing therapies lack durable efficacy and the remaining high unmet medical need for novel therapies for CLBP leads the

Sponsor to believe that the risk/benefit assessment is positive for subjects to be exposed to PP353 in the Persica 002 study.

2. Study Objectives

The specific objectives are:

Objectives	Part A	Part B
Primary	- To evaluate the safety and tolerability of PP353 when administered by intradiscal injection - To characterise the PK profile of a single dose of PP353 in plasma to enable a choice of dosing regimen to be used in Part B to give approximately 8 days of intradiscal exposure to PP353 as inferred from plasma exposure	- To evaluate the safety and tolerability of PP353 when administered by intradiscal injection - To evaluate the efficacy of PP353 as measured by change from baseline in LBP NRS score at 12 months
Secondary	-	- To characterise the PK profile of PP353 in plasma for the dosing regimen selected in Part A to give approximately 8 days of intradiscal exposure to PP353 in the active arm as inferred from plasma exposure - To evaluate the efficacy of PP353 as measured by change from baseline in LBP score at 3, 6 and 9 months - To assess the efficacy of PP353 as measured by improvement in disability due to CLBP

Exploratory Part A and Part B	- To evaluate the relationships between drug exposure, efficacy and subject reported outcomes - To explore imaging biomarkers related to quantitative assessment of inflammation and oedema in the spine - To characterise the PK profile of selected excipients from dose(s) of PP353 in plasma (analysis will depend on the stability of the PK samples already collected and the consent of the subjects if consent has not already been obtained for such analysis) - To investigate if systemic (blood) cytokine levels measured at baseline is related to response to treatment.	
Exploratory Part A	- To evaluate the efficacy of PP353 as measured by change from baseline in LBP NRS score at 3, 6, 9 & 12 months - To assess the efficacy of PP353 as measured by improvement in disability due to CLBP	
Exploratory Part B		- To evaluate the efficacy of PP353 as measured by change from baseline in average LBP daily score over a 7-day period at 3, 6, 9 & 12 months

3. Study Design

3.1 Description of Study

This is a placebo-controlled, two-part study, each part of 12 months' duration, in subjects with CLBP associated with vertebral body endplate bone oedema as detected by MRI. In both parts of the study, the subject population will not have been previously treated with antimicrobial agents for their back pain.

Part A of the study is open label to evaluate the safety, tolerability and PK of PP353 in subjects with CLBP associated with vertebral body oedema as detected by MRI.

Part B of the study is a randomised, placebo-controlled, investigator and subject-blinded, third-party unblinded study to evaluate the safety, tolerability and efficacy of two administrations of PP353 given approximately 4 days apart in the above subjects.

Part A

The aim of Part A is to determine the injection regimen for Part B and establish the safety and tolerability of a single injection of PP353.

Subjects in Part A will receive only one injection of up to 3 mL of PP353, a total linezolid dose of 150 mg, delivered directly into the disc and intervertebral disc space under fluoroscopy guidance.

Subjects with longer than 6 months of CLBP and vertebral body endplate bone oedema who have had an inadequate response to current standard of care will be screened for inclusion in Part A. An inadequate response to current standard of care is defined as having a residual pain score of ≥ 4 on chronic pain medication and ≥ 6 if not on chronic pain medication on the LBP NRS and a RMDQ-23 score of ≥ 9.

Subjects will provide signed informed consent and be screened for inclusion in the study. Consent must be obtained prior to any study specific procedure.

Screening may occur over a period of up to 28 days (Days -28 to -1) prior to dosing on Day 1. Where a washout period is required for any contraindicated or prohibited medications, the washout period may commence prior to the start of the 28-day screening window. If a subject has not had a standard of care MRI, a suitability MRI (without gadolinium), may be conducted after a subject has signed consent. A suitability MRI may be performed prior to the start of the 28-day screening window.

All other screening procedures must be performed within the protocol specified window for screening.

Eligible subjects in Part A will be admitted for the drug administration procedure and remain in hospital for an overnight period and should remain in a recumbent (on bed rest) position for a period of 8 hours after administration. After this time subjects will be advised to undertake only modest activity levels that do not provoke pain for the first 14 days following injection, and only engage in activity within pain limits thereafter.

Subjects in Part A of the study will have an MRI, including macrocyclic gadolinium (Gd)-enhanced contrast MRI at baseline, 6 months and 12 months participation (or early termination) in the study.

MRI data will be collected to measure Modic changes and assess changes in bone oedema and inflammation in the spine.

However, it is recognised that conventional MRI does not allow for the assessment of functional tissue characteristics such as diffusion and perfusion. Adding DCE-MRI to the acquisition protocol will ensure that the volume and level of inflammation are captured at the

baseline and assessed after the treatment providing a valuable opportunity to assess quantitative inflammation driven changes in the spine. A range of reproducible and sensitive quantitative biomarkers will be extracted from the DCE-MRI sequences. These imaging biomarkers will allow assessment of the early impact of the treatment on the vascularity of tissue and correlation of these to the clinical outcome measures.

Subjects will be followed for 12 months to evaluate the safety, tolerability and clinical benefit of PP353.

The first three subjects in Part A will be dosed sequentially with a gap of at least 14 days between each subject being administered the injection. It is intended that an intradiscal administration of PP353 will provide a depot effect to provide a high local concentration significantly above the MIC. Systemic (blood) sampling will be performed over the next 10 days to establish the PK profile of PP353. Plasma PK will be used as a surrogate marker for the intradiscal depot as direct intradiscal sampling is not possible.
As each subject completes the Day 11 visit (end of PK sampling), all samples for that subject will be shipped to the bioanalytical lab for immediate analysis and reporting.
Safety, tolerability and PK data from these first three subjects will be analysed and reviewed by the SRC and a decision will be made as to whether the PK data are consistent enough to determine a choice of regimen for Part B. If the PK data are not consistent, then a further three subjects can be enrolled into Part A to obtain additional data for analysis and review by the SRC to reach a decision on a choice of regimen for Part B. The second set of three subjects can be dosed sequentially or in parallel depending on the recruitment rate.
A detailed description of the procedures, data flow, and meeting schedule of the SRC are provided in the SRC charter.
The decisions to proceed to Part B and the number of injections to be administered in Part B should be based on, but not limited to, the following safety aspects:

- The safety of PP353 when administered as an intradiscal injection, as evaluated by all adverse events, laboratory and ECG abnormalities.
- The tolerability of intradiscal administration of PP353, which will be evaluated by pain scores and subject discomfort.
- The duration of intradiscal exposure of PP353, which will be assessed and inferred by plasma PK analysis of PP353 administration. While the linezolid depot remains in the disc, plasma linezolid concentration is expected to be in steady state. When the depot

runs out, the plasma linezolid concentration is expected to enter exponential decline. When plasma linezolid concentration falls below 5 ng/mL, the intradiscal linezolid concentration is expected to be approximately 10 µg/mL. This is the target trough concentration that would lead to the next PP353 injection if required to maintain intradiscal exposure for approximately 8 days.

Part B

Part B is a randomised, investigator- and subject-blinded, third-party (pharmacist and drug administrator) unblinded, placebo-controlled study of up to 40 subjects. Subjects will be randomised to one of two arms testing active versus placebo for safety, tolerability and efficacy of PP353.

Arm 1: Active treatment

Twenty subjects treated with two intradiscal injections of PP353 on Day 1 and Day 5. Each intradiscal injection will be up to 3 mL of PP353, a linezolid dose of 150 mg, delivered directly into the intervertebral disc under contrast guidance.

Arm 2: Placebo

Twenty subjects treated with two placebo injections. Subjects on placebo will receive two sham injections, with the skin pierced and the needle penetrating the deep fascia, stopping 2 to 3 cm short of the intervertebral disc.

The regimen in Part B was determined following Part A and will consist of 2 intradiscal injections administered over a period of 5 days to give approximately 8 days exposure to PP353 within the intervertebral disc.

As the regimen for Part B consists of more than one intradiscal injection, a sentinel pair (blinded; one subject on active and one subject on placebo) will be dosed followed by the rest of the cohort no sooner than 14 days after the last injection of the sentinel pair.

Subjects with longer than 6 months of CLBP and vertebral body endplate bone oedema who have had an inadequate response to current standard of care will be screened for inclusion in Part B. An inadequate response to current standard of care is defined as having a residual pain score of ≥ 4 on chronic pain medication and ≥ 6 if not on chronic pain medication on the LBP NRS and a RMDQ-23 score of ≥ 9.

Subjects will provide signed informed consent and be screened for inclusion in the study.

On confirmation of eligibility, subjects will be randomised to active or placebo treatment.

In Part B, subjects will be admitted for each injection procedure and remain in hospital for at least 8 hours after each procedure. Following each injection, the subject must have limited activity for at least 8 hours in order to maximise the opportunity for the study medication to remain within the intervertebral disc space; they should remain lying down on bed rest, e.g. recumbent, semi-recumbent or on their side, for the first 4 hours and then have limited activity for the subsequent 4 hours after each injection as described in the Procedure Manual. Due to the injection some subjects may experience post procedural pain requiring an overnight stay. Post procedural pain requiring a single overnight stay in hospital will not be reported as an SAE (unless other SAE criteria are also fulfilled). Post procedural pain will be captured as an adverse event and summarised separately in the CSR. Subjects will be advised to undertake only modest activity levels that do not provoke pain between injections, for the first 14 days following the last injection and only engage in activity within pain limits thereafter.
Participants in Part B will have an MRI examination, at baseline, Month 6 and Month 12/ early termination; DCE-MRI sequences should be done on all subjects where possible. In cases where the DCE-MRI procedure cannot be conducted by the site or the subject is unable to tolerate the procedure, the DCE-MRI sequence can be omitted. However, all other sequences should be performed. If the baseline DCE-MRI has not been conducted, then DCE-MRI should not be performed at the 6 or 12 month/early termination visit. The acquisition of a DCE-MRI sequence at 6 months was ratified by the SRC who reviewed the utility of the data from the available scans from subjects enrolled in Part A of the study and available Part B screening MRIs. Specifications for acquisition and analysis of DCE-MRI are provided in the Imaging Review Charter and Imaging Manual.

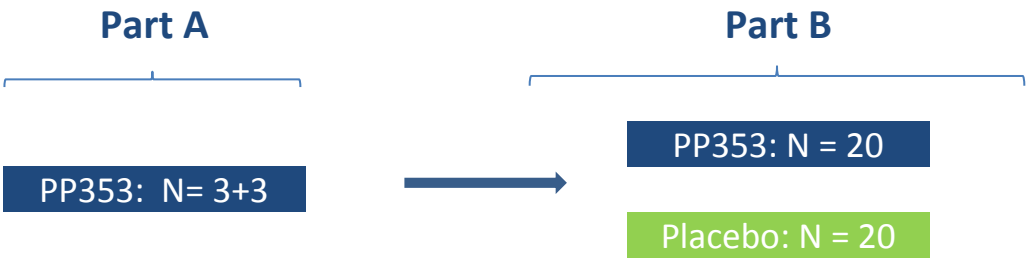
Subjects will be treated with two injections to provide intradiscal exposure of PP353 for approximately 8 days and will then be followed for 12 months to evaluate the safety, tolerability and clinical benefit of PP353.

Overall, the results of this trial will be used for further development of PP353.

3.1.1 Overview of Study Design

The study overview is presented in Figure 1.

Figure 1: PP353 Study Overview



3.1.2 Safety Review Committee

The SRC will be responsible for review of data and for making the decision in Part A whether the PK data are consistent enough to determine a choice of regimen for Part B. The incidence and nature of any adverse events, serious adverse events, and laboratory abnormalities will be assessed on an ongoing basis by the SRC. The SRC will consist of designated Sponsor representatives, (including but not limited to the contracted medical monitor, medical consultant, pharmacovigilance, biostatistics, clinical pharmacology), as well as any other individuals whom the Sponsor requires to assist with such assessments. When necessary, an SRC member may be replaced at any meeting by a designee.

A detailed description of the procedures, data flow, and meeting schedule of the SRC are provided in the SRC charter.

3.1.3 Stopping Rules

The SRC must temporarily halt or stop the study if either of the following scenarios occur:

1. Any '**serious**' adverse reaction considered related to the study drug in two or more subjects during the study.
2. Any '**severe**' non-serious adverse reaction considered related to the study drug in three or more subjects during the study.

If the study is stopped, no further subjects will be dosed and ongoing subjects will continue to be followed up as per the protocol. If the study is temporarily halted, recruitment may re-start after any recommendations of the SRC have been implemented and any necessary amendments approved by the regulatory authorities and ethics committees.

Stopping Rules for Part A or Part B:

The SRC will stop dosing in Part A if either of the following scenarios occurs in Part A with a reasonable possibility of a causal relationship to PP353. Similarly, the SRC will stop dosing in Part B if either of the following scenarios occurs in Part B with a reasonable possibility of a causal relationship to PP353:

- Two or more subjects have a confirmed QTcB > 500 msec and/or an increase of > 60 msec from baseline on repeated ECGs performed ≥ 2 min apart
- Two or more subjects have hepatic toxicity defined as one of the following:
    - Confirmed increase in alanine aminotransferase (ALT) or aspartate aminotransferase (AST) to > 3x upper limit of the laboratory normal range (ULN) or a doubling from baseline where the subject has a baseline value which is greater than 1.5x ULN
    - Confirmed isolated increase in total bilirubin to > 2x ULN or a doubling from baseline where the subject has a baseline value which is greater than the ULN
    - Confirmed increase in ALT and/or AST to > 3x ULN in conjunction with an increase in total bilirubin to > 2x ULN in the absence of cholestasis (serum alkaline phosphatase < 2x ULN)
    - Confirmed increase in ALT and/or AST to > 5x ULN in the absence of a concomitant bilirubin elevation

Any non-serious adverse reactions will be reviewed in aggregate to assess the number of subjects in which they occur and concurrency of more than one in the same subject. Following the review of data, it may be considered appropriate to stop further dosing in a Part A or Part B or potentially the study.

If a subject in the study experiences any of the above (i.e., conditions of sufficient severity that risk would exceed benefit), the investigator must immediately notify the Sponsor, who will suspend all dosing in the study and convene a meeting of the SRC as soon as possible following knowledge of the event.

In Part A, if after a review of the study data, the SRC unanimously agrees with the investigator's original assessment that the event would meet stopping criteria, further dosing within the entire study must be suspended until the SRC consults with the Persica senior management team (PSMT).

In Part B, if after a review of the study data, the SRC unanimously agrees with the investigator's original assessment that the event would meet stopping criteria if the subject

had received active drug, the subject's treatment will be unblinded to the SRC. If the subject did receive PP353, further dosing within the entire study must be suspended until the SRC consults with the PSMT.

3.2 Length of Study and End of Study

The total duration of this study for each subject is approximately 13 months (Part A) and 14 Months (Part B), including screening, enrolment, treatment, and follow-up.

The study will consist of the following:

- Screening and enrolment/randomisation: Subjects may be screened for eligibility up to 28 days (Part A) and 56 Days (Part B) prior to enrolment/randomisation (Day -28/-56 to Day -1).

Consent must be obtained prior to any study specific procedure. Where a washout period is required for any contraindicated or prohibited medications (Part A), consent may be obtained and the washout period may commence before the start of the 28 day screening window. If a subject has not had a standard of care MRI, a suitability MRI (without gadolinium), may be conducted after a subject has signed consent. Sites may administer a suitability assessment of the RMDQ-23 questionnaire and Low Back Pain Numeric Rating Scale/Leg Pain Numeric Rating Scale to avoid sending ineligible subjects for suitability MRIs. These suitability assessments are not databased. A suitability MRI, suitability RMDQ-23 and suitability Low Back Pain Numeric Rating Scale/Leg Pain Numeric Rating Scale may be performed prior to the start of the 28-day (Part A) or 56-day (Part B) screening window.

All other screening procedures (including the screening RMDQ-23 and Low Back Pain Numeric Rating Scale/Leg Pain Numeric Rating Scale) must be performed within the protocol specified window for screening (unless the Sponsor has agreed an extension).

- Treatment and Follow-up:
  - Part A: On Day 1, subjects will be admitted to hospital for the intradiscal injection and will remain in hospital overnight following the injection administration procedure. Subjects will return to the study site on Days 5, 7, 9 and 11 for safety assessments and collection of PK samples.
  - Part B: In Part B, subjects will be admitted to the hospital for the first intradiscal injection and will remain in hospital for at least 8 hours following the injection administration procedure. Subjects will return to the study site for one further injection on Day 5 and will remain in hospital for at least 8 hours following the second injection administration procedure. Thereafter, follow up visits are scheduled

for safety assessments and collection of PK samples as detailed in the schedule of assessments (see Appendix A).

- In both parts A and B, all subjects will be followed until Month 12 (Day 365) to evaluate the safety, tolerability and clinical benefit of PP353 (evaluation of clinical benefit in Part A is exploratory only). Following the intervention period, subjects will have follow-up visits at Months 1, 3, 6, 9 and 12.

Refer to the Appendix A: Schedule of Activities.

Subjects who discontinue the study early for whatever reason will be encouraged to complete all the Month 12 (Day 365) visit assessments as an early termination / end of study visit.

Subjects who are unable to return to the study centre to attend follow-up visits are to be contacted by phone to: 1) determine health status, 2) record adverse event information, and 3) ask that the diary, LBP NRS, ODI and RMDQ-23 questionnaires be completed.
The end of the study is defined as the first day when all subjects have had a study completion visit, early termination visit or have otherwise been discontinued from the study.

3.3 Rationale for the Study Design

3.3.1 Rationale for PP353 Dose and Schedule

Refer to Section 1.5 for the discussion of dose selection.

Part A

Subjects in Part A will receive one injection of up to 3 mL of PP353, a total linezolid dose of 150 mg, delivered directly into the intervertebral disc under contrast guidance.

Part B

Subjects in Part B will be randomised to receive either PP353 or placebo. The regimen in Part B was determined following Part A and will consist of two intradiscal injections administered on Day 1 and Day 5 to give approximately 8 days' exposure to PP353 within the intervertebral disc.

Active: PP353 - each active injection will be up to 3 mL of PP353, a linezolid dose of 150 mg, delivered directly into the intervertebral disc under contrast guidance.

Placebo: Placebo injections are described in Section 3.1.

Part B	Dose (PP353/Placebo)	Number of Subjects
Arm 1	Two injections of up to 3 mL of 50 mg/mL of linezolid suspension of PP353	20
Arm 2	Two placebo injections	20

3.3.2 Rationale for Subject Population

As discussed in the Introduction, there is strong scientific evidence that CLBP may be due to intradiscal bacterial infection and given the lack of efficacy of many of the available pharmaceutical or surgical options, the unmet medical need remains high. Furthermore, previous studies with oral antibiotics (see Section 1.1) have provided benefit in similar subject populations. This study will assess the safety, tolerability, efficacy and PK of PP353 in up to 40 subjects with > 6 months of CLBP and vertebral body endplate bone oedema who have had an inadequate response to current standard of care. The lower age of inclusion (18 years) was selected since feedback from study investigators indicates that there are subjects with Modic changes in this age bracket with significant unmet medical need. The upper age has been selected (70 years) to limit the potential for alternative pathologies/comorbidities ongoing concurrently which may make interpretation of the results of this proof of concept/efficacy difficult to delineate in a small number of subjects.
The inclusion and exclusion criteria have been designed to safeguard the subjects from undue risk and to minimise comorbidities that could confound the interpretation of adverse outcomes.

Given that the current CLBP treatment regimens are largely ineffective, the unmet medical need for alternative therapies is high, and socioeconomic costs associated with this condition are high, subjects with CLBP are an appropriate subject population for evaluation with this medicinal product.

3.3.3 Rationale for Placebo Control

This study will include a placebo treatment arm in Part B in order to evaluate the safety, and clinical effects of PP353 in subjects with CLBP and vertebral body endplate bone oedema. Placebo is an appropriate comparator to test the hypothesis in this proof of concept study. As described in the Introduction, many of the existing therapies for CLBP are ineffective and the

courses of antibiotics referenced above are not approved for the treatment of CLBP and are prolonged (100 days' therapy).

All subjects will continue to receive their usual standards of care, with the exception of prohibited medications as detailed in Section 4.4 (Concomitant Therapy)

3.4 Endpoints

3.4.1 Primary Endpoints

3.4.1.1 Safety and Tolerability – Part A and B

Safety and tolerability will be assessed based on the incidence of AEs throughout the duration of the study.

3.4.1.2 Pharmacokinetics – Part A Only

The following PK parameters will be determined for plasma linezolid:

- Single dose (data collected from Day 1 to last sample): T_{dur} ,t_{max}, AUC_{0-t}, C_{max},
- If possible, other parameters will be determined such as $t_{1/2}$, $AUC_{0-\infty}$, CL/F.

Sampling scheme for Part A is presented in Appendix A.

3.4.1.3 Efficacy – Part B

Efficacy will be assessed by the change from baseline of LBP NRS score at 12 months.

The LBP NRS will be used to capture subject-reported low back pain intensity.

Low back pain intensity now, worst low back pain intensity in the last 14 days and average low back pain intensity in the last 14 days will be assessed by the subject on an 11-point scale with 0 = "no pain" and 10 = "the worst possible pain you can imagine." The average of the three scores will be used to summarise the subject's low back pain.

3.4.2 Secondary Endpoints

3.4.2.1 Pharmacokinetics – Part B

PK parameters (T_{dur} ,t_{max}, $AUC_{0-\tau}$, $C_{max,}$ $t_{1/2}$, CL/F) will be determined for plasma linezolid as secondary endpoints for Part B.

Sampling scheme:

The final PK blood sampling scheme adopted for Part B was determined based on analysis of data from Part A. The blood samples to be collected will be restricted to a maximum total of 16 samples of 4 mL. Sampling scheme for Part B is presented in Appendix A.

3.4.2.2 Efficacy – Part B

Efficacy for Part B will be assessed using the following:

- Change from baseline of LBP NRS score at 3, 6 and 9 months. LBP NRS scores will be calculated as described in 3.4.1.3.
- RMDQ is a widely used health status measure for low back pain and is designed to assess self-rated physical disability caused by low back pain (Roland *et al*. 2000).
  - The change from baseline in RMDQ-23 score at 3, 6, 9 and 12 months will be assessed.
  - Clinically relevant improvement at 3, 6, 9 and 12 months, defined as ≥ 30% reduction from baseline in the RMDQ-23 score will be assessed.
- The ODI is derived from the completed Oswestry Low Back Pain Questionnaire (Fairbank *et al*., 2000). Change from baseline in ODI at 3, 6 and 12 months will be evaluated.

3.4.3 Exploratory Endpoints

3.4.3.1 Safety and Tolerability – Part A and Part B

Safety and tolerability will be assessed based on the incidence of abnormal findings in clinical laboratory tests (serum chemistry, haematology, urinalysis), physical examinations, vital signs, and electrocardiograms (ECGs).

3.4.3.2 Efficacy – Part A and Part B

The following subject-reported outcomes and DCE-MRI and/or MRI assessments will be evaluated at all timepoints assessed (see Schedule of Activities, Appendix A):

- Change from baseline in hours with low back pain during the last 4 weeks. This will be derived by multiplying the number of days with low back pain (over the last 4 weeks) with the average number of hours per day with low back pain (over the last 4 weeks)
- Subject global perceived effect

- Change from baseline of leg pain NRS score.
  Leg pain NRS score: A numeric rating scale will be used to capture subject reported leg pain intensity. Leg pain intensity now, worst leg pain intensity in the last 14 days and average leg pain intensity in the last 14 days will be assessed by the subject on an 11-point scale with 0 = "no pain" and 10 = "the worst possible pain you can imagine."
  The average of the three scores will be used to summarise the subject's leg pain.
- Change from baseline in use of analgesic medication. This will include the number of days that analgesic medication was taken due to low back pain, during the week prior to each visit; specific drug and dosage will also be collected.
- Change from baseline in RMDQ-24 score. The additional questions required to assess RMDQ-24 are included and answered at each occasion that RMDQ-23 is completed.
- Change from baseline in the volume and severity of vertebral body endplate oedema and inflammation, as captured by DCE-MRI and/or MRI.

In addition, cytokine levels at baseline will be determined and may be evaluated for any correlation with response to treatment. The efficacy endpoints to be included in this analysis will be detailed in the SAP.

3.4.3.3 Pharmacokinetics – Part A and B

The following PK parameters may be determined for selected excipients as exploratory endpoints for Part A and B (analysis will depend on the stability of the PK samples already collected and the consent of the subjects if consent has not already been obtained for such analysis):

- Single dose (data collected from Day 1 to last sample): t_{max}, AUC_{0-t}, C_{max},
- If possible, other parameters will be determined such as $t_{1/2}$, $AUC_{0-\infty}$, CL/F.

3.4.3.4 Efficacy – Part A Only

The following variables will be assessed as exploratory efficacy endpoints in Part A:

- Change from baseline in LBP NRS score at 3, 6, 9 & 12 months
- Change from baseline in RMDQ-23 score at 3, 6, 9 and 12 months
- Clinically relevant improvement at 3, 6, 9 and 12 months, defined as ≥ 30% reduction from baseline in the RMDQ-23
- Change from baseline in ODI at 3, 6 and 12 months

3.4.3.5 Efficacy – Part B Only

The following variables will be assessed as exploratory efficacy endpoints in Part B:

- Change from baseline of average LBP intensity NRS daily score over a 7-day period at 3, 6, 9 & 12 months
  Each question will be assessed by the subject on an 11-point scale with 0 = "no pain" and 10 = "the worst possible pain you can imagine." For the 7 days prior to the scheduled visits, subjects will record the number that best describes their average low back pain over the past 24 hours.

3.5 Randomisation and Blinding

Subjects selected for participation in Part A of the study meet the inclusion and exclusion criteria and are therefore representative of the population who will be selected to participate in Part B of this protocol. The subject population identified in this protocol are generally representative of the CLBP population with age and intercurrent disease limitations and are appropriate to a proof of concept study at this stage of development.

Part A of the study is open label and all subjects will receive active PP353. The purpose of this part of the study is to primarily determine the safety, tolerability and PK of PP353. The PK assessment is objective and not subject to bias. Subjects who complete Part A of the protocol will be analysed separately to the randomised, placebo-controlled subjects in Part B.

Part B of the study is a placebo-controlled, investigator and subject-blinded, third-party (injector and pharmacy) unblinded because of the impracticality of blinding PP353 for injection due to differences between the active and placebo injections.
The randomisation list will not be available to the subjects, investigators, (blinded) monitors, or employees of the clinical centres involved in the management of the study before unblinding of the data, unless in case of emergency.
The Sponsor's clinical team will not have direct access to the randomisation list. However, those that are unblinded members of the SRC will have access to unblinded data as detailed in the Sponsor Blinding Plan and SRC Charter. Individual data, excluding subject identification numbers, may be looked at by parties independent of the project team should there be a corporate business requirement to do so.

The SRC will be blinded to safety and efficacy data; unblinded members of the SRC will have access to unblinded data as detailed in the SRC Charter.
The contract research organisation (CRO) performing blinded monitoring, data management and statistical activities will receive a copy of the randomisation list after database lock.
Other blinded team members will not have access to any data that could lead to unblinding. A separate unblinded monitoring team will be responsible for drug accountability and related monitoring activities in Part B of the study.
The steps taken by the study teams to maintain the blind will be documented in a blinding plan.

Each participating site will be required to draw up a blinding plan to document the processes to be followed at a site to ensure that the blinded site staff members are not unblinded to treatment assignment.

Allocation of treatment will be performed by interactive response technology (IRT) which will allocate a randomisation number and treatment allocation to each subject when they have passed the selection criteria.
Unblinding of the individual subject’s treatment by the investigator should be limited to medical emergencies or urgent clinical situations in which knowledge of the subject's study treatment is necessary for clinical management. Once a subject's treatment assignment has been unblinded, the medical monitor and study coordinator should be notified within 24 hours of unblinding of the treatment. Information relating to unblinding (e.g., reason and date) shall be clearly recorded in the subject’s study file. In addition, the investigator should consider whether the clinical event prompting unblinding should be considered a serious adverse event (SAE), according to the regulatory definitions or criteria for SAEs, and if so, submit an SAE report to Persica or designee.
Persica, the SRC or designee will also unblind any SAE reports that are serious, unexpected, and considered to be related to the study drug, in accordance with safety reporting guidance and regulations.

4. Materials and Methods

4.1 Trial Population

4.1.1 Number of Subjects and Description of Population

Part A is an open label cohort of up to six subjects. The first three subjects will be enrolled and treated in a staggered fashion. If a further three subjects are required to determine the dosing regimen for Part B, the next three subjects may be enrolled in parallel.
Part B is a randomised, investigator and subject-blinded, third-party unblinded, placebo-controlled study of up to 40 subjects.
Subjects who were enrolled in Part A cannot enrol in Part B.

4.1.2 Subject Numbering

At screening, upon signing the informed consent form (ICF), subjects will be assigned a unique subject screening number. Eligible subjects who are enrolled in the trial will be assigned a unique subject randomisation number for treatment assignment.
The trial site will maintain a list identifying all subjects by their identification numbers and year of birth.

4.2 Eligibility Criteria

Exceptions for eligibility criteria will not be permitted during the trial, either by the investigator or by the Sponsor study physician.
Consent must be obtained before any study specific procedures are performed.

Medications (Part A) where a washout period is required for any contraindicated or prohibited medications, consent may be obtained and the washout period may commence before the start of the 28 day screening window.

If a subject has not had a standard of care MRI, a suitability MRI (without gadolinium), may be conducted after a subject has signed consent.

Sites may administer a suitability assessment of the RMDQ-23 questionnaire and Low Back Pain Numeric Rating Scale/Leg Pain Numeric Rating Scale to avoid sending ineligible subjects for suitability MRIs. These suitability assessments are not databased.

A suitability MRI, suitability RMDQ-23 and suitability Low Back Pain Numeric Rating Scale/Leg Pain Numeric Rating Scale may be performed prior to the start of the 28-day (Part A) and 56-day (Part B) screening window.

All other screening procedures (including screening RMDQ-23 questionnaire and Low Back Pain Numeric Rating Scale/Leg Pain Numeric Rating Scale) must be performed within the protocol specified window for screening (unless the Sponsor has agreed an extension).

4.2.1 Inclusion Criteria

To be eligible to participate in this study, all the following criteria must be met by all subjects at the timepoints indicated:

	Inclusion Criteria	Screening	Day 1 pre-randomisation
1.	Can understand and sign the written informed consent form (ICF).	X	
2.	Considered reliable and capable of adhering to the protocol.	X	X
3.	Aged between 18 and 70 years, inclusive.	X	
4.	CLBP in the area L1 to S1 associated with vertebral body endplate bone oedema (Modic 1) or vertebral body endplate bone oedema and fat (Modic 1 and 2) at a single lumbar level. *Modic 1 or Modic 1 and 2 are allowed at non-lumbar levels e.g., cervical. Modic 2 is allowed at levels more than 2 vertebrae away from the target with the limitations described in exclusion 3.1. Modic 3 (Subchondral bone sclerosis) is permitted at any level.*	X[1]	X[2]
5.	Current episode of CLBP has lasted for ≥ 6 months at the time of randomisation.	X	X
6.	Average LBP NRS score (from 14-day recall question, Appendix B Subject Questionnaire) at screening and at Day 1 pre-randomisation ≥ 4 on chronic pain medication and ≥ 6 if not on chronic pain medication; it should be higher than the leg pain NRS score. *Chronic pain medication is defined as taking analgesia on at least 4 days out of 7 in the week prior to screening and the week prior to randomisation. Exceptions to this criterion are permissible following discussion between the Sponsor study physician and investigator.*	X	X

7.	RMDQ-23 score ≥ 9 at screening and at Day 1 pre-randomisation.	X	X
8.	Central review of imaging confirms the disc changes are suitable for the study and there are no other significant spinal pathologies that could account for the pain symptoms.		X
9.	Bodyweight of ≥ 50 kg and ≤ 120 kg. Subject is able to undergo required protocol procedures and in the opinion of the investigator, the target lumbar disc is likely to be injected successfully. *Exceptions to this criterion are permissible following discussion between the Sponsor study physician and investigator.*	X	X
10.	Failure of standard of care therapies used by their treating physician	X	
11.	Agrees to use highly effective contraception*:		
a.	Female subjects of child-bearing potential must agree to use dual methods of contraception (including one highly effective and one effective method of contraception*) from the start (signing consent) until 1 month after the end of the study and have a negative serum pregnancy test at screening.	X	
b.	Male subjects must use an acceptable effective* barrier method of contraception if sexually active with a female of child-bearing potential from the start of the study until 100 days after the last injection.	X	
c.	Male participants agree not to donate sperm until 100 days after the last injection.	X	

**Highly effective and acceptable effective methods of contraception are described in Section 5.2.10 of the protocol.*
[1.] Based on historical or suitability MRI, if available
[2.] Eligibility MRI

4.2.2 Exclusion Criteria

Subjects are not permitted to enrol in the study if any of the following criteria are met:

		Screening	Day 1 Pre-randomisation
1.	No longer applicable.	NA	NA
2.	The target lumbar disc has lost more than half its original anticipated height at the centre or it is < 5mm in height over the central 15 mm portion.	X[1]	X[2]

	Exceptions to this criterion are permissible in cases where the investigator believes the target disc is accessible to injection and following discussion between the Sponsor study physician and investigator.		
3.	No longer applicable.	NA	NA
3.1	Any vertebra with Modic 2 only lesions which: a) in the opinion of the investigator, after deep palpation of the vertebral spine, is contributing to the low back pain and/or b) are present within 2 vertebrae from the target lumbar disc. *For clarification: If the target disc is L5/S1, Modic 2 in the L3 vertebra would be permissible provided the investigator does not think that lesion is contributing to the back pain.*	X[1]	X[2]
4.	Gross facet joint degeneration or cases where the investigator believes the primary pain generator to be the facet joints.	X[1]	X[2]
5.	In the investigator's clinical judgement, there is a clear alternative cause for back pain, e.g. inflammatory joint disorders.	X	
6.	Therapeutic interventional back procedure in the 6 months (26 weeks) prior to screening, with the exception of corticosteroid injections which are excluded in the 3 months (13 weeks) prior to screening. Diagnostic procedures or procedures which only provide transient pain relief e.g. epidural injection, are permitted but the Investigator should allow sufficient time (at least 4 weeks) after the diagnostic procedure for the subject's pain to have settled back to their normal levels before screening.	X	
7.	History of alcohol abuse or drugs of abuse within the past two years.	X	
8.	No longer applicable.	NA	NA
9.	No longer applicable.	NA	NA
9.1	Taking any prohibited medications. *See section 4.4.2*	X[3]	X[3]
10.	No longer applicable.	NA	NA
11.	Unable to temporarily stop using anticoagulant or anti-platelet treatment around the time of the injection. The use of low dose aspirin (up to and including100 mg per day) is permitted. *See section 4.4.2*		X[3]
12.	Poorly controlled diabetes mellitus type 1 and type 2. A poorly controlled diabetic defined as having an HbA1c level greater than 64 mmol/mol (8%) in the preceding 6 months will be excluded from the study.	X	

13.	Poorly controlled hypertension.	X	
14.	Female subject who is pregnant or plans to become pregnant during the study, or is breastfeeding.	X	X
15.	Received any investigational drug or experimental procedure to treat their low back pain within 180 days prior to screening.	X	
16.	Previously been treated with antimicrobial agents for their low back pain or previously received any antimicrobial intradiscal injection.	X	
17.	Active infection (e.g., sepsis, pneumonia, abscess) in addition to the suspected chronic discal infection. Or has taken a course of antibiotic treatment for > 14 days within 6 months prior to study drug administration. *When in doubt, the investigator should confer with the Sponsor study physician and in certain cases the subject may be included.*	X	X
18.	Required to take or likely to take prophylactic antibiotic therapy for whatever reason over the duration of the study. The use of prophylactic antibiotic administration in the peri-surgery period, other than linezolid, is permitted as per institution policy.	X	X
19.	No longer applicable.	NA	NA
20.	No longer applicable.	NA	NA
21.	Platelet count of < 100 x 10^9/L.	X[4]	
22.	White cell count < 3.0 x 10^9/L (3000/mm^2) or absolute neutrophil count (ANC) < 2.0 x10^9/L (2000/mm^2).	X[4]	
23.	Reduced kidney function, defined as an eGFR of < 45 mL per min per 1.73 m^2, calculated by the Modified Diet in Renal Disease (MDRD) study equation. *Subjects with eGFR of 45-60 mL per min per 1.73m2 can be included in the study but* ***must not*** *receive gadolinium during any study MRIs.*	X[4]	
24.	Renal or liver impairment, defined as:		
a.	For women, serum creatinine level ≥ 125 μmol/L; for men, ≥ 135 μmol/L, or	X[4]	
b.	Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) ≥ 1.5 x upper limit of normal (ULN), or alkaline phosphatase (ALP) and/or bilirubin > 1.5 x ULN. *Subjects with a diagnosis of Gilberts disease with bilirubin up to 3 x ULN may be included after discussion and agreement with the Sponsor study physician.*	X[4]	
25.	HIV positive, history of or current hepatitis, or carriers of HBsAg and/or anti HCV.	X	

26.	Active neoplastic disease or history of neoplastic disease within 5 years of screening (except for basal or squamous cell carcinoma of the skin or carcinoma *in situ* that has been definitively treated with standard of care).	X	
27.	Progressive visual loss or macular degeneration. Closed angle glaucoma or progressive cataract occlusion are permitted.	X	
28.	Major surgery within the previous 12 weeks prior to screening. Major surgery is defined as surgery requiring general anaesthesia, or a surgery that lasts longer than 30 min, is not performed laparoscopically or is not for a trivial dermal procedure (e.g., wart removal or excision of benign lesion/tumour).	X	
29.	Impaired cardiac function or clinically significant cardiac diseases, including any of the following:		
a.	Unstable angina or acute myocardial infarction ≤ 12 weeks prior to Screening;	X	
b.	Clinically significant heart disease (e.g. symptomatic congestive heart failure [e.g. more severe than New York Heart Association [NYHA] Class 2]; uncontrolled arrhythmia,).	X	
30.	Serious psychiatric or medical conditions including but not limited to: subjects with phaeochromocytoma; carcinoid; thyrotoxicosis; bipolar depression; schizoaffective disorder; acute confusional states that, in the opinion of the investigator, could interfere with treatment, compliance, or the ability to give consent.	X	
31.	History of hypersensitivity or allergies to linezolid, poloxamer 407 gel, iohexol or iodine; or documented intolerance to required medication for the procedure e.g. local anaesthetics (lignocaine, bupivicaine and marcaine) or systemic anaesthetics e.g propofol.	X	
32.	Acute clinically significant illness within 30 days prior to study drug administration.		X
33.	Contraindication to MRI examination including, but not limited to, intracranial metal clips, heart pacemakers, insulin pumps, implanted hearing aids, neurostimulators, metal hip replacements, profound claustrophobia, or inability to lie in the MRI machine in an appropriate position to obtain quality images. *Subjects with a history of allergic reactions to gadolinium may be enrolled in the study but* ***must not*** *receive gadolinium during any study MRIs.*	X	
34.	Subjects who do not have suitable veins for multiple venepunctures/cannulation as assessed by the investigator at screening	X	

35.	Subjects with significant medical comorbidities that, in the opinion of the investigator, should not participate in the trial.	X	X
36.	Participation in any other clinical research study within 90 days prior to screening and for the duration of Persica 002.	X	

[1] *Based on historical or suitability MRI, if available*

[2] *Eligibility MRI*

[3]*Review concomitant medications at screening for contraindicated and prohibited medications*

[4]*If local laboratory tests are performed pre-surgery as routine practice, any clinically significant abnormalities detected are to be reported as an adverse event. If any of the local lab results are exclusionary, the investigator must discuss with the Sponsor study physician prior to dosing.*

Subjects may be re-screened for inclusion following written approval from the Sponsor. If the subject is re-screened a new ICF must be signed, a new screening number assigned, and all screening procedures repeated. Please note that the screening MRI should NOT be done until it has been confirmed that the subject is eligible on all other criteria. In the case of a subject screen failing after having already had a screening MRI scan, then the subject can be rescreened without the need to repeat the study MRI scan, provided rescreening occurs within 12 weeks of the study MRI scan.

Tests may be repeated during the screening period with the written approval of the Sponsor if for example, the subject has an out-of-range laboratory value which in the opinion of the investigator is not clinically significant.

4.2.3 Method of Treatment Assignment and Blinding

Once a subject has fulfilled the entry criteria, he/she will be assigned a unique identifier (randomisation number) by the interactive response technology (IRT). The unblinded IMP co-ordinator, unblinded pharmacist or properly trained unblinded designee will randomise the subject using IRT.

All subjects enrolled in Part A will be assigned PP353.

Subjects in Part B will be assigned to PP353 or placebo randomly in a 1:1 ratio.

4.3 Study Treatment

The investigational medicinal product (IMP) for this study is PP353.

4.3.1 Formulation, Packaging, and Handling

PP353 has been formulated to allow intervertebral disc injection of the active ingredient linezolid, a diluent containing poloxamer 407 to enable delivery and a contrast agent (iohexol) to enable dosing under image guided injection.
The test materials for this study will be supplied by the study Sponsor and are as follows:

- PP353-A (milled linezolid API): a white to yellowish powder in 10-mL clear light amber glass vials. Store refrigerated (2–8°C). PP353-A will be supplied in boxes of six uniquely numbered vials per box.

- PP353-B (diluent for suspension): A light-sensitive, clear, colourless to pale yellow, viscous liquid, with no visible particulates, not less than 7 mL in 10-mL clear glass vials. Store refrigerated (2–8°C) protected from light when not in use and ensure exposure to light is minimised during use. Supplied in boxes of six uniquely numbered vials per box.

The drug product PP353 will undergo suspension using aseptic technique at the pharmacy or at the site of drug administration (e.g., surgery) of the investigational sites. During suspension and transfer between pharmacy and site of administration, time under ambient conditions must be minimised and the drug product must be kept at 2–8°C until just prior to administration. Drug product must be used within 3 hours of final mixing. Detailed instructions for suspension are provided in the Persica Study 002 Pharmacy Manual.

4.3.2 Dosage and Administration

Part A: open label
Subjects in Part A will receive one injection of up to 3 mL of PP353, a linezolid dose of 150 mg, delivered directly into the intervertebral disc under contrast guidance.

Part B:
The regimen in Part B was determined following Part A and will consist of two intradiscal injections administered under contrast guidance on Day 1 and Day 5 to give approximately 8

days' exposure to PP353 within the intervertebral disc. Subjects will be randomly assigned to receive PP353 or placebo at a 1:1 ratio:

Part B	Dose (PP353/Placebo)	Number of Subjects
Arm 1 - active	Two injections of up to 3 mL of 50 mg/mL of linezolid suspension of PP353	20
Arm 2 - placebo	Two placebo injections	20

Administration

PP353 will be injected directly into the affected intervertebral disc using aseptic technique and image guidance.

The injection into the disc may be painful and all subjects will require systemic and/or local anaesthetic during the procedure and analgesia in the immediate post-operative period. Guidance for administration of anaesthetic procedures is provided in the Procedure Manual. All of the anaesthetics administered must be reported in the electronic case report form (eCRF) as concomitant medication.

The subject must be alert, conscious and responsive as the needle is being introduced past the nerve root to minimise any potential risk of damage to the associated nerve root.

Subjects receiving placebo will undergo the same anaesthetic procedures as the subjects on the active arm.

In Part A, on completion of the administration of PP353, the subject should be observed according to the local site practice and be returned to the ward for an overnight stay. In Part B, on completion of the administration of PP353/Placebo, the subject should be observed according to the local site practice for a minimum of 8 hours before discharge.

Adverse events associated with the procedure will be recorded in the subject medical records and reported in the eCRF.

Instructions for administration of the active and placebo injections are provided in the Procedure Manual.

Imaging of injection procedures will be transmitted to a central imaging review centre and reviewed by an independent reviewer as directed by the SRC.

4.3.3 Investigational Medicinal Product Accountability

The study site will acknowledge receipt of IMP and confirm the shipment condition and content. Any damaged shipments will be replaced.

The IMP is to be stored in a secure pharmacy or locked area, with access limited to authorised personnel, in accordance with the details provided in the investigator's brochure and Pharmacy Manual. Upon receipt, PP353-A and PP353-B vials must be refrigerated at 2–8°C until use.

Accurate records of all IMPs received at, dispensed from, returned or disposed of by the study site are to be recorded on the drug accountability records.

Documentation of individual subject drug suspension and drug accountability records must be kept as described in the Pharmacy Manual.

If allowed by site pharmacy policy, used vials of PP353-A and PP353-B should be retained by the site pharmacy until drug accountability has been verified by the responsible study Monitor, following which, used IMP vials will either be disposed of at the study site according to the study site's institutional standard operating procedure or returned to the Sponsor with the appropriate documentation. The site's method of IMP destruction must be agreed to by the Sponsor. The site must obtain written authorisation from the Sponsor before any IMP is destroyed, and IMP destruction must be documented on the appropriate form.

4.3.4 Post-trial Access

This is the first in human trial of PP353. The safety and efficacy of PP353 have not been proven in this indication. As such, the Sponsor does not intend to provide PP353 to subjects after the conclusion of the study or any earlier withdrawal. All subjects who complete this study or drop out of the study should consult their treating physicians to receive the usual standard of care for their CLBP.

4.4 Concomitant Therapy

4.4.1 Permitted Concomitant Medications

The use of any concomitant medication/therapy, including over-the-counter (OTC) medications deemed necessary for the care of the subject is permitted during the study provided they do not have a known antimicrobial or immunosuppressive effect (refer to 4.4.2).

Any concomitant medications used during the study must be documented in the subject records and recorded in the eCRF.

The administration of an antibiotic in an acute situation should not be withheld but given if clinically warranted and discussed with the Sponsor study physician afterwards. A course of antibiotic treatment is defined as > 14 days.

The use of prophylactic antibiotics, other than linezolid, is permitted in the peri-surgical period, as per institutional policy; this should be recorded in the eCRF. All subjects should receive antibiotic cover for the administration of study treatment as per the institutional policy.

Introductions of medications which have analgesic activity e.g., amitriptyline for depression should be avoided where possible. If such medications are required to be started during the study, alternative non-analgesic medications should be considered if clinically acceptable.

Hormonal contraceptives are permitted in women of child-bearing potential. Hormonal contraceptives include any marketed contraceptive agent that includes an oestrogen and/or a progestational agent.

COVID-19 vaccinations take priority over receiving PP353/Placebo. The Sponsor has conducted a risk assessment of the administration of PP353/Placebo and the concomitant administration of a COVID-19 vaccine. No interaction between any of the currently licensed COVID-19 vaccines and PP353 is anticipated.
However, as this is a Phase 1b study, the Sponsor wishes to avoid adverse events that are related to COVID-19 vaccinations over the injection period.

- Patients should receive their first injection procedure at least 7 days after receiving a COVID-19 vaccine.
- Patients should not receive a COVID-19 vaccine in the 14 days after their last injection procedure.

Vaccinations against COVID 19 (or any other organism), should be reported as a concomitant medication in the eCRF. Symptoms related to vaccinations should be reported as adverse events.

Any diagnostic, therapeutic, or surgical procedure performed during the study period should be recorded in the eCRF, including the dates, description of the procedure(s), and any clinical findings, if applicable.

4.4.2 Prohibited Concomitant Medications

The use of antimicrobials or immunosuppressive agents is restricted in the study. If clinically required, the use of any antimicrobial or immunosuppressive agents must be discussed by the investigator and the medical monitor on a case-by-case basis.

Oral or IV administration of linezolid should be avoided for 14 days prior to first injection procedure and up to 14 days after the last injection procedure.

COVID-19 vaccines should be avoided for 7 days prior to first injection procedure and up to 14 days after the last injection procedure.

Anti-coagulants and anti-platelet treatments, other than low dose aspirin (up to and including 100mg/day), are contraindicated at the time of the injection due to increased bleeding risk. If clinically appropriate, these can be stopped prior to first injection procedure and then restarted after the last injection procedure.

4.5 Study Assessments

Appendix A: Schedule of Activities details the timing of study visits and the assessments to be performed at each visit.
Consent must be obtained prior to any study specific procedure. Where a washout period is required for any contraindicated or prohibited medications (Part A), consent may be obtained and the washout period may commence before the start of the 28 day screening window. If a subject has not had a standard of care MRI, a suitability MRI (without gadolinium), may be conducted after a subject has signed consent.

Sites may administer a suitability assessment of the RMDQ-23 questionnaire and Low Back Pain Numeric Rating Scale/Leg Pain Numeric Rating Scale to avoid sending ineligible subjects for suitability MRIs. These suitability assessments are not databased.

A suitability MRI, suitability RMDQ-23 and suitability Low Back Pain Numeric Rating Scale/Leg Pain Numeric Rating Scale may be performed prior to the start of the 28-day (Part A) and 56-day (Part B) screening window.

All other screening procedures (including screening RMDQ-23 questionnaire and Low Back Pain Numeric Rating Scale/Leg Pain Numeric Rating Scale) must be performed within the protocol specified window for screening (unless the Sponsor has agreed an extension).

4.5.1 Informed Consent Forms and Screening Log

Written informed consent for participation in the study must be obtained from all subjects in their native or preferred language before any trial related procedures (including any study specific screening procedures) are performed. The Subject Information and ICF will be approved by the same independent ethics committee (IEC) that approves this protocol. Each ICF will comply with the ICH-GCP guidelines and local regulatory requirements.
The investigator will ensure that the Sponsor reviews and authorises any site specific ICF used in the trial before submission to the IEC.
Investigators may discuss trial availability and the possibility for entry with a potential subject without first obtaining consent. However, informed consent must be obtained and documented prior to initiation of any procedures that are performed solely for the purpose of determining eligibility for research, including withdrawal from current medication(s).
Once appropriate essential information has been provided and fully explained in layman's language to the subject by the investigator (or a medically qualified designee), and it has been documented that the subject has had the opportunity to ask questions, the IEC-approved ICF will be signed and dated by both the subject and the person obtaining consent (investigator or designee), as well as by any other parties required by the IEC. The subject will receive a copy of the signed ICF; the original shall be kept on file by the investigator. Additional copies will be filed according to local requirements.
Potential subjects are free to refuse entry into the trial or withdraw from the trial at any time, without justification, without any consequences to their further care.
Subjects may be asked to sign additional ICFs if the protocol is amended and the changes to the protocol results in additional information that needs to be provided to the subjects, so that they can make a knowledgeable and voluntary decision on trial participation.
The investigator will maintain a screening log to record details of all subjects screened and to confirm eligibility or record reasons for screening failure, as applicable.

4.5.2 Medical History and Demographic Data

At screening, all available medical records of the subject will be reviewed and a comprehensive medical history will be collected from the subject including history of CLBP and collection of medication and procedures specific to the treatment of CLBP. Medical history includes clinically significant diseases and procedures, including chronic diseases, infections, surgeries, cancer history (including prior cancer therapies and procedures), reproductive status, smoking history, use of alcohol and drugs of abuse, and all medications (e.g., prescription drugs, over-the-counter drugs, herbal or homeopathic remedies, nutritional supplements) used by the subject. Report treatments for CLBP for a period of at least 1 year, and other treatments for a period of at least 3 months, prior to the screening visit.

At each subsequent study visit, a review of concurrent medical conditions will be performed and any new conditions or clinically significant worsening of medical history conditions are to be recorded as adverse events on the Adverse Event eCRF.

Demographic data will include year of birth and sex.

4.5.3 Safety Assessments

4.5.3.1 Physical Examinations

At screening, a complete physical examination must be performed. Any abnormality identified at baseline should be recorded on the Medical History eCRF.

At subsequent visits (or as clinically indicated), an abbreviated, symptom-directed physical examination should be performed at the times shown in Appendix A: Schedule of Activities. Changes from baseline abnormalities should be recorded in subject records. New or worsened clinically significant abnormalities are to be recorded as adverse events on the Adverse Event eCRF.

4.5.3.2 Vital Signs

Vital signs will be taken at the times shown in Appendix A: Schedule of Activities, , vital signs will include measurements of respiratory rate, pulse rate, temperature (oral or tympanic), and systolic and diastolic blood pressures; measurement will be taken once the subject has been seated or supine position for at least 5 min.

Height and weight will be recorded at screening and at Month 12. Weight will also be recorded at Day 1 Pre dose.

4.5.3.3 Clinical Laboratory Tests

The clinical laboratory tests listed in Table 4.5.3.3-1 will be collected at the times shown in Appendix A: Schedule of Activities, and processed in accordance with directions from the clinical laboratory.

Table 4.5.3.3-1 - Clinical Laboratory Tests

Haematology: Haemoglobin Haematocrit Red cell count Mean cell volume Mean corpuscular haemoglobin concentration Platelet count White cell count Neutrophils Lymphocytes Monocytes Eosinophils Basophils Urinalysis (Local Testing): URINE TEST STRIP If clinically significant abnormalities are detected on urine dipstick site to perform local follow-up and management. Additional Tests: (Central Laboratory) HBsAg anti-HCV HIV 1 and 2 antibodies Serum pregnancy test (All female subjects at screening. All female subjects at Day 30, unless permanently surgically sterile) Additional Tests: (Local testing) Urine pregnancy test will be conducted prior to administration of each intradiscal injection and before each MRI in female subjects (unless permanently surgically sterile).	Serum Chemistry: Alkaline phosphatase Alanine transferase Aspartate transferase Bilirubin Blood urea nitrogen (optional dependent on capability of central laboratory) Calcium Chloride Creatinine Gamma-glutamyl transferase Lactic dehydrogenase Potassium Protein, total Sodium Urea Uric acid Triglycerides / Fasting triglycerides* Creatine kinase Albumin Globulin Magnesium Phosphate Random blood glucose (SST) / Fasting blood glucose (SST)* Cholesterol/Fasting Cholesterol* HDL cholesterol/Fasting HDL cholesterol * LDL cholesterol/Fasting LDL cholesterol * Estimated Glomerular Filtration Rate (MDRD equation)

* *Site staff to record fasting status on the laboratory requisition form at the time of collection of the blood samples*

4.5.3.4 Electrocardiograms

Single 12-lead ECG recordings will be obtained at specified timepoints, as shown in Appendix A: Schedule of Activities, and may be obtained at unscheduled timepoints as described below. ECG recordings are to be printed out in duplicate for subject medical records to permit collection of an original tracing by the Sponsor, if required.

All ECG recordings must be performed using a standard high-quality, high-fidelity digital electrocardiograph machine equipped with computer-based interval measurements. Lead placement should be as consistent as possible. ECG recordings must be performed after the subject has been resting in a supine position for at least 10 min. All ECGs are to be obtained prior to other procedures scheduled at that same time (e.g., vital sign measurements, blood draws) and if possible, should not be obtained within 3 hours after any meal. Circumstances that may induce changes in heart rate, including environmental distractions (e.g., television, radio, conversation) should be avoided during the pre-ECG resting period and during ECG recording. ECGs recorded on thermosensitive paper to be photocopied for subject records to retain legibility.

For safety monitoring purposes, the investigator or designee must review, sign, and date all ECG tracings and indicate if any abnormalities are clinically significant. Paper copies of ECG tracings will be kept as part of the subject's permanent records at the site. The following should be recorded in the appropriate eCRF: heart rate, RR interval, QRS duration, PR interval, uncorrected QT interval, and QT interval corrected (QTcB) based on the machine readings of the individual ECG tracings. Any morphologic waveform changes or other ECG abnormalities must be documented on the eCRF. If considered appropriate by the Sponsor, ECGs may be analysed retrospectively at a central laboratory.

If, at a post-dose timepoint, the mean corrected QT interval is > 500 ms and/or > 60 ms longer than the baseline value, another ECG must be recorded, ideally within the next 5 min, and ECG monitoring should continue until the corrected QT interval has stabilised on two successive ECGs. The Sponsor Study Physician should be notified. Standard of-care treatment may be instituted per the discretion of the investigator. The investigator should also evaluate the subject for potential concurrent risk factors (e.g., electrolyte abnormalities, comedications known to prolong the QT interval, severe bradycardia).

4.5.4 Efficacy Assessments

4.5.4.1 Subject Reported Outcomes

Subject reported outcomes shown in the table below will be collected at the timepoints specified in Appendix A: Schedule of Activities. Parameters collected at screening will be recorded in a paper questionnaire completed by the subject during the visit. All subsequent timepoints will be recorded in an diary (electronic or paper):

Outcome	Timepoint
• Low back pain and leg pain: NRS assessment of low back and leg pain intensity: Low back pain/leg pain intensity now, worst low back pain/leg pain intensity in the last 14 days and average low back pain/leg pain intensity in the last 14 days will be assessed by the subject on an 11-point scale with 0 = "no pain" and 10 = "the worst possible pain you can imagine". The average of the three scores will be used.	• Screening • Day 1, pre-dose • Part A – Day 5 • Months 1, 3, 6, 9, and 12
• Hours with lower back pain during the last 4 weeks	• Screening • Pre-dose on Day 1 • Months 1, 3, 6, 9, and 12
• Subject global perceived effect starting at the day 30 visit	• Months 1, 3, 6, 9, and 12
• Average LBP intensity NRS daily score (Part B subjects only)	• Day 1 - pre-dose and Month 12: each day for 7 days leading up to the actual planned visit day as determined by the site • Month 1, 3, 6, 9: each day for 7 days leading up to the scheduled visit day as derived from the date of the first injection
• Analgesic use for low back pain	At screening the subject will trained on use of the diary to report analgesic use for low back pain. The subject will record analgesic medication taken for low back pain in the diary • Day 1 - pre-dose and Month 12: each day for 7 days leading up to the actual planned visit day as determined by the site • Month 1, 3, 6, 9: each day for 7 days leading up to the scheduled visit day as derived from the date of the first injection

4.5.4.2 Roland-Morris Disability Questionnaire

RMDQ-23 is a self-administered disability measure. In addition to the 23 questions assessed in the RMDQ-23, a further five questions related to low back pain disability will be completed and used with a subset of the RMDQ-23 questions to derive the RMDQ-24 score. The RMDQ-23 and five additional disability questions will be completed at screening and at Day 1 pre-dose, Months 1, 3, 6, 9 and 12 (or Early Termination).

Refer to Appendix C: Roland-Morris Disability Questionnaire (RMDQ-23) + additional disability questions.

4.5.4.3 Oswestry Disability Index

The ODI is derived from a subject-completed questionnaire which gives a subjective percentage score of level of function (disability) in activities of daily living in those rehabilitating from low back pain. The questionnaire examines perceived level of disability in 10 everyday activities of daily living.
The ODI will be completed at Day 1 pre-dose, Months 3, 6 and 12 (or Early Termination).
Refer to Appendix D: Oswestry Disability Index.

4.5.4.4 Magnetic Resonance Imaging

MRI, DCE-MRI will be obtained during the screening period, at Month 6 (Day 180) and Month 12 (Day 365) / Early Termination.

DCE MRI remains mandatory for all subjects in Part A.

In Part B, DCE-MRI sequences should be done on all subjects where possible. In cases where the DCE-MRI procedure cannot be conducted by the site or the subject is unable to tolerate the procedure, the DCE-MRI sequence can be omitted. However, all other sequences should be performed. **If the screening DCE-MRI has not been conducted, then DCE-MRI should not be performed at the 6 or 12 month visit.**

Subjects with a screening eGFR of 45-60 mL per min per 1.73m2 or with a history of allergic reactions to gadolinium can be included in the study but **must not receive gadolinium during any study MRIs**.

If a subject has not had a standard of care MRI, a suitability MRI (without gadolinium), may be conducted after a subject has signed consent. A suitability MRI may be performed prior to the start of the 28-day (Part A) and 56-day (Part B) screening window.

The Screening MRI, DCE-MRI scan is to be performed within the screening window, but only after all other screening tests have confirmed subject eligibility. It is recommended to allow at least 7 days between the date of screening MRI, DCE-MRI and Day 1. This is to allow for central MRI eligibility confirmation prior to randomisation.
Central MRI eligibility confirmation must be obtained prior to randomisation.
If an MRI fails QC at central review, due to **gadolinium sequences only**, it does not need to be repeated.

If the screening MRI is not available with acceptable gadolinium sequences, then the 6-month and 12-month MRI scans should be done **without gadolinium**.

If an MRI fails QC at central review due to a poor-quality image, the **non-gadolinium** sequences that failed QC may be repeated.

If the screening MRI fails QC due to a poor-quality image, and the screening window cannot be met, the subject will need to be re-screened per protocol, with the exception that the repeat MRI should be done **without gadolinium**.

If a subject fails screening having already had an acceptable screening MRI, then the subject can be re-screened **without the need to repeat the screening MRI scan**, provided re-screening occurs within 12 weeks of the screening MRI scan.

Other than any suitability MRI, which will remain at the site, all MRI scans will be pseudonymised at the site and pseudonymised data will be transmitted to a central facility for reading and reporting once any personal identifiers have been removed.

The decision to continue acquiring DCE-MRI at 6 months in Part B, was made by the SRC following review of the quantitative DCE-MRI biomarkers from the scans acquired during Part A of the study and available Part B screening MRIs,

Other than any suitability MRI, acquisition of MRI scans will be performed according to the Imaging Manual.

4.5.5 Pharmacokinetic Assessments

Blood samples for PK analysis will be collected at the timepoints presented in the Schedule of Activities in Appendix A.

If a PK sample cannot be drawn at the designated time, an acceptable window for each sample collection is described in Appendix A; the exact date and time of the draw must be recorded in the source and in the eCRF.

Samples will be analysed for concentrations of linezolid and may be analysed for concentrations of selected excipients.

Plasma samples will be collected, prepared and shipped to the bioanalytical laboratory. Detailed handling and shipping instructions are provided in the laboratory manual.

4.5.6 Cytokine analysis

Baseline (Day 1 pre-dose) blood samples that have been collected for PK analysis may be analysed for concentrations of selected cytokines including IL-13, where additional consent has been obtained.

Cytokine testing may include (but is not limited to); IFN-gamma, IL-1 beta, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12 p70, IL-13 and THF-alpha.

4.6 Subject, Treatment, Study, and Site Discontinuation

4.6.1 Subject Discontinuation

Subjects have the right to voluntarily withdraw from the study at any time for any reason. In addition, the investigator has the right to withdraw a subject from the study at any time.

Reasons for withdrawal from the study may include, but are not limited to, the following:

- Subject withdrawal of consent at any time
- Any medical condition that the investigator or Sponsor determines may jeopardise the subject's safety if he/she continues in the study
- Investigator or Sponsor determines it is in the best interest of the subject

Every effort should be made to obtain information on subjects who withdraw from the study. Subjects should be encouraged to have a final study visit/Early termination visit wherever possible. The primary reason for withdrawal from the study should be documented on the appropriate eCRF. However, subjects will not be followed for any reason after consent has been withdrawn.

4.6.2 Study Treatment Discontinuation

Subjects must discontinue study treatment if they experience a significant SAE and if, in the opinion of the investigator, it may place the subject in significant risk of harm.

The primary reason for study treatment discontinuation should be documented on the appropriate eCRF.

4.6.3 Study and Site Discontinuation

The Sponsor, investigator, or the IEC has the right to terminate the participation of a trial site, if necessary, due to lack of subject enrolment, noncompliance with the protocol, or if judged to be necessary for medical, safety, regulatory, ethical, or other reasons consistent with

applicable laws, regulations, and GCP. The Sponsor is to be notified promptly if the trial was terminated by the investigator or the IEC at the trial site.

4.6.4 Study Completion/Early Termination

Subjects who complete all study visits up to and including Month 12 (Day 365 +/- 7 days) are considered to have completed the study. All subjects who discontinue from the study early will be asked to complete all assessments for Month 12 / Early Termination. Please refer to Appendix A: Schedule of Activities for the assessments to be performed at the Early Termination visit.

5. Assessment of Safety

5.1 Safety Plan

PP353 is not approved and is currently in clinical development, thus the safety profile is not known at this time. It should be noted that this is the first clinical study with this formulation of PP353, and although linezolid, poloxamer and iohexol have all been approved for human use, they have not been evaluated in combination. As such the safety profile for this formulation of PP353 has not been established. No Serious Adverse Reactions (SARs) are considered expected by the Sponsor for the purpose of expedited reporting of Suspected Unexpected Serious Adverse Reactions (SUSARs) for the Investigational Medicinal Product (IMP).

The safety plan is designed to ensure subject safety and will include specific eligibility criteria and monitoring assessments as detailed below.

The investigator, in consultation with the Sponsor, is responsible for assuring the safety of study participants who have entered this study and for taking appropriate action concerning any event that seems unusual, even if this event may be an unanticipated benefit to the study participant. The investigator will be responsible for a clinical assessment of the study participants before discharge from the study, and for the establishment of a discharge plan, if needed.

Administration of PP353 or placebo will be performed in a setting with emergency medical equipment and personnel who are trained to monitor for and respond to medical emergencies. A qualified physician will administer the intradiscal injections under image guidance.

In Part A, subjects will be admitted to hospital for the first intradiscal injection and will remain in hospital overnight following the injection administration procedure. Subjects are to

remain in a recumbent position (on bed rest) for 8 hours post dose, undertake only modest activity levels that do not provoke pain for the first 14 days following injection, and only engage in activity within pain limits thereafter.

In Part B, subjects will be admitted for each injection procedure and will remain in hospital for at least 8 hours after each procedure. Following each injection, the subject must have limited activity for at least 8 hours in order to maximise the opportunity for the study medication to remain within the intervertebral disc space; they should remain lying down on bed rest, e.g. recumbent, semi-recumbent or on their side, for the first 4 hours and then have limited activity for the subsequent 4 hours after each injection as described in the Procedure Manual. Due to the injection some subjects may experience post procedural pain requiring an overnight stay. Post procedural pain requiring a single overnight stay in hospital will not be reported as an SAE (unless other SAE criteria are also fulfilled). Post procedural pain will be captured as an adverse event and summarised separately in the CSR. Subjects will be advised to undertake only modest activity levels that do not provoke pain between injections, for the first 14 days following the last injection and only engage in activity within pain limits thereafter.

During the study, the incidence and nature of AEs, SAEs, and laboratory abnormalities will be assessed. A blinded review of safety will be performed on an ongoing regular basis by the SRC as described in the SRC charter.

5.2 Adverse Event Reporting

5.2.1 Collection Period

Adverse events, treatment-emergent adverse events, serious adverse events and pregnancies will be reported from the time the subject gives signed consent to the final visit.

5.2.2 Definitions

Adverse Event

An AE is any untoward medical occurrence in a subject administered a medicinal (investigational or non-investigational) product in a clinical study. An AE does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational or non-investigational)

product, whether or not related to that medicinal (investigational or non-investigational) product.

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, or abnormal results of diagnostic procedures, including clinical laboratory test abnormalities.

If there is an exacerbation of chronic lower back pain at the level of interest beyond what is reasonably expected by the treating physician this will be reported as an adverse event.

Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose meets any of the following conditions:

- results in death
- is life-threatening, i.e., the subject was at risk of death at the time of the event (e.g., ventricular fibrillation and anaphylaxis). The term does not refer to an event which hypothetically might have caused death if it were more severe.
- requires in-subject hospitalisation or prolongation of existing in-subject hospitalisation: Hospitalisation refers to an overnight admission into hospital for the purpose of investigating and/or treating the AE. Hospitalisation for an elective procedure, or routinely scheduled treatment for a pre-existing condition that has not worsened, is not an SAE.

  For operational purposes, should a subject have administration of PP353 late in the day or be required to stay overnight in the hospital due to post procedural pain or study required observations, this will not be considered as an SAE (unless other SAE criteria are also fulfilled), however these AEs will be collected and summarised separately in the clinical study report.
- results in persistent or significant disability/incapacity, i.e., causing substantial disruption of the subject's ability to conduct normal life
- is a congenital anomaly/birth defect. Congenital abnormalities and birth defects in the offspring of male or female subjects should be reported as an SAE if conception occurred during the study treatment period or in the 100-day period after the last dose of study drug.
- is medically significant, i.e., may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the subject's health or may require intervention to prevent one of the above outcomes. Examples of such events are intensive treatment in an

emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalisation or development of drug dependency or drug abuse.

Unexpected Adverse Event

An AE is considered unexpected if the nature or severity is not consistent with the applicable product reference safety information (Investigator's Brochure).

Suspected Unexpected Serious Adverse Reactions

SUSARs are SAEs that are unexpected and judged by the investigator or Sponsor to be related to the study treatment administered.

The Sponsor or designee will report SUSARs and other applicable SAEs to the appropriate regulatory authorities, ECs and investigators as required, according to local law.

Treatment-Emergent Adverse Event

A treatment-emergent adverse event is defined as any event not present prior to the initiation of the treatments or any event already present that worsens in either intensity or frequency following exposure to the treatments. Any adverse events which are reported after the signing of the informed consent to the time of administration of PP353 are pre-treatment adverse events and will be reported and analysed separately.

5.2.3 Intensity of Adverse Events

Each AE must be rated on a 5-point scale of increasing intensity according to the Common Terminology Criteria for Adverse Events version 4.0:

Note: the semi-colon within the description of the grade indicates 'or'.

Grade 1:

Mild: asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2:

Moderate: minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily life (preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.).

Grade 3:

Severe or medically significant but not immediately life-threatening: hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self-care activities of daily life

(bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).

Grade 4:

Life-threatening consequences: urgent intervention indicated.

Grade 5:

Death related to AE.

5.2.4 Causality Assessment

The following binary choice will be used by the investigator to describe the causality assessment with the test treatment:

Related

There is evidence to suggest a causal relationship between the test treatment and the AE.

Unrelated

There is no evidence to suggest a causal relationship between the test treatment and the AE.

5.2.5 Action Taken Regarding Investigational Product

The action taken towards the study drug must be described as follows:

No action

Permanently discontinued

Stopped temporarily

5.2.6 Outcome

The outcome of each AE must be rated as follows:

- Recovered/resolved
- Recovering/resolving
- Not recovered/not resolved
- Recovered with sequelae/resolved with sequelae
- Fatal
- Unknown

5.2.7 Injection Site Reactions

Subjects should be monitored after each injection at 30 min, 1 h, 8 h and, for Part A only, immediately before discharge for any injection site reaction. In Part B, “immediately before

discharge" injection site reaction assessments need to be performed only if the subject has remained in hospital for more than 12 h after an injection procedure. The occurrence of any injection site reaction should be monitored until resolved.

Injection site reactions will be recorded in the eCRF and assessed according to the scales below.

Any injection site reaction where one or more symptom is reported, must be reported as 'injection site reaction' in the AE log with the start date (and time, if starting on a dosing day), stop date, causality and severity. Severity of the AE is to be recorded as, at a minimum, the highest severity of the symptoms recorded, unless assessed to be more severe in the clinical judgement of the investigator.

Injection Site Reaction Grading Scale

Location Reaction	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 h or interferes with activity	Any use of narcotic pain reliever or prevents daily activity
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest
Erythema/redness	2.5 – 5 cm	5.1 – 10 cm	> 10 cm
Induration/swelling	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity

5.2.8 Recording of Adverse Events

All (S)AEs occurring during the clinical investigation must be documented in the EDC system.

Whenever possible, diagnoses should be given when signs and symptoms are due to a common aetiology (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as "upper respiratory infection"). Investigators must record their opinion concerning the relationship of the (S)AE to the study drug in the EDC system. All measures required for (S)AE management must be recorded in the source documents and reported according to Sponsor's instructions.

If a patient is tested positive for COVID-19 (or any other infection) at any time after signing informed consent, an adverse event must be reported in the eCRF, even if they are asymptomatic. Negative tests do not need to be reported.
Subjects who have tested positive for COVID-19 should NOT be withdrawn from the study.

All (S)AEs occurring at any time during the study (including the follow-up period) will be followed by the investigator until satisfactory resolution (e.g., value back to baseline value) or stabilisation or until final database lock.
Pregnancies in subjects or female partners of male subjects will be reported as described in 5.2.10. Pregnancies should be followed to first well-baby visit. If necessary, in order to obtain additional information to ensure safety to the subject, additional blood and urine samples may be taken at the discretion of the investigator. Congenital abnormalities and birth defects in the offspring of male or female subjects should be reported as an SAE if conception occurred during the study treatment period or in the 100-day period after the last dose of study drug.
Certain long-term AEs related to therapy cannot be followed until resolution within the setting of this study. Any AE which is not resolved at the time of database lock should be denoted as ongoing. In these cases, follow up will be the responsibility of the treating physician.

5.2.9 Reporting of Serious Adverse Events

All SAEs, irrespective of the circumstances or suspected cause, must be reported on a Serious Adverse Event Form by the investigator to the Sponsor or designee as soon as possible and within 24 hours of their knowledge of the event, by fax or by scanned email.
The Sponsor and medical monitor will be notified of SAEs within 24 hours.

Contact details for reporting SAEs:
Refer to the Investigator Site File for reporting contact details.

The SAE form should include a clearly completed narrative describing signs, symptoms, and treatment of the event, diagnostic procedures, as well as any relevant laboratory data and any sequelae, in order to allow a complete medical assessment of the case and independent determination of the possible causality.
Follow-up and outcomes should be reported for all subjects that experience an SAE.

It is critical that the information provided on the Serious Adverse Event Form matches the information recorded in the EDC system for the same event. In addition, the same information is to be recorded in the source documents.
Copies of additional reports and documents should be sent when requested and applicable.
Follow-up reports relative to the subject's subsequent course must be submitted to CRO until the event has subsided or, in case of permanent impairment, until the condition stabilises.

5.2.10 Pregnancy

Subjects should not become pregnant during the study.
The investigator must report any pregnancy which occurs in a female subject for the duration of the study or the female partner of a male subject up to 100 days after last dose by emailing the pregnancy form to the Sponsor or designee within 24 hours of the study site staff becoming aware of the pregnancy. Pregnancies should be followed to first well-baby visit. The Investigator or study site staff must report the outcome of the pregnancy to the Sponsor or designee.
Contact details for reporting pregnancies: Refer to the Investigator Site File for reporting contact details.

Congenital abnormalities and birth defects in the offspring of male or female subjects should be reported as an SAE if conception occurred during the study treatment period or in the 100-day period after the last dose of study drug.

Highly effective and acceptable effective methods of contraception
Birth control methods which may be considered as highly effective are those that can achieve a failure rate of less than 1% per year when used consistently and correctly.
Such methods include:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
    - oral
    - intravaginal
    - transdermal
- progestogen-only hormonal contraception associated with inhibition of ovulation:
    - oral
    - injectable

  - implantable
- intrauterine device
- intrauterine hormone-releasing system
- bilateral tubal occlusion
- vasectomised partner
- sexual abstinence (The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject)

Acceptable birth control methods are those that result in a failure rate of more than 1% per year and include:

- progestogen-only oral hormonal contraception, where inhibition of ovulation is not the primary mode of action
- male or female condom with or without spermicide
- cap, diaphragm or sponge with spermicide

5.2.11 Reporting of SAEs to Competent Authorities / Ethics Committees

Persica or designee is responsible for appropriate reporting of AEs to the regulatory authorities. Persica or designee will also report to the investigator all SAEs that are unexpected and associated with the use of the drug. The investigator must report these events to the appropriate IEC that approved the protocol, unless otherwise required and documented by the IEC.

Persica or designee, will be responsible for unblinding and submitting SUSARs involving the study drug to the applicable regulatory authorities according to ICH guidelines. In addition, Persica or designee, will be responsible for the submission of safety letters to the relevant ECs and to participating investigators of all SUSARs involving PP353 according to applicable regulations. For clinical sites that use a local EC, it is the responsibility of the investigator to promptly notify the local EC of all unexpected serious adverse drug reactions according to the EC requirements.

After completion of the clinical study, determined as last patient, last visit (LPLV), any unexpected safety issue that changes the risk–benefit analysis and is likely to have an impact on the subjects who have participated in it, will be reported by the CRO as soon as possible to the competent authority(ies) concerned together with proposed actions.

6. Statistical Considerations and Analysis Plan

The database will be cleaned on an ongoing basis and locked when all subjects have completed the study (Month 12) or discontinued. The results of the two study parts will be analysed separately.

The primary objective of this study is to characterise the safety, tolerability and efficacy of PP353 compared with placebo when administered in subjects with CLBP. Statistical summaries will include means, standard deviations, and percentiles for continuous variables or frequency counts and percentages (using number of subjects in the analysis set as the denominator) for categorical variables). Subjects will be grouped according to treatment received.

The number of subjects who enrol, discontinue (early discontinuation of treatment or early termination from the study), and complete the study will be tabulated by treatment group using descriptive statistics. Reasons for early discontinuation of treatment or early termination from the study will be listed and summarised by treatment group. Any eligibility criteria exceptions and other protocol deviations will be reported by treatment group.

Demographic and baseline characteristics of the subject will be summarised by treatment group by use of descriptive statistics. Baseline is defined as the last available value prior to study drug administration. Subject disposition, concurrent treatment, and compliance with study treatment and visits will be summarised using descriptive statistics.

Cytokine analyses will be detailed in the SAP.

6.1 Definition of Analysis Sets

The enrolled set (ES) will consist of all subjects who give informed consent for Persica 002, including screen failures.

The randomised set (RS) is applicable to Part B only and will consist of all subjects who were randomised into the Persica 002 study.

The safety set (SS) will consist of all enrolled subjects who receive at least one dose of PP353.

The full analysis set (FAS) will consist of all enrolled subjects who receive at least one dose of PP353 and have a valid post-baseline measurement for at least one efficacy variable.

The per protocol set (PPS) is a subset of the FAS, consisting of those subjects who had no important protocol deviations that may affect the validity of the efficacy data. If warranted, selected efficacy outputs may be repeated for the PPS.

6.2 Determination of Sample Size

Part A: No formal power calculation was performed for Part 1. The sample size of three subjects with the possibility of additional three subjects is considered sufficient for the PK analyses for the choice of regimen for Part B.

The decision to use an additional three subjects will be evaluated on:

- the safety and tolerability data of the first three individuals,
- the variability of the pharmacokinetics in the first three subjects, and an assessment of whether the data are enough to provide a reliable pharmacokinetic profile and
- whether the PK data provide enough information to confirm the PK sample timepoints in Part B.

Part B: Power calculations were based on the results of the large CLBP randomised control trial (Albert *et al*., 2013b). That study's database was used to select the subset of subjects with a baseline RMDQ-23 score of ≥ 9 and a baseline LBP NRS score of ≥ 4 that is also higher than the leg pain NRS score to fit the inclusion criteria. In this subset of subjects, the subjects receiving oral antibiotics had a mean reduction from baseline in the LBP rating scale at 1 year of 3.28 units and placebo subjects had a reduction of 0.61 units. A conservative reduction of 1.2 units for the placebo group, as estimated from the AIM study (Bråten et al., 2019) was used rather than a reduction of 0.61 units in the final sample size calculation.

A sample size of 20 subjects per arm (allowing 20% for withdrawals) is considered sufficient to detect a difference in CLBP reduction between the active and placebo groups of at least 2.08 units, assuming a common standard deviation of 2.3. A one-sided, two-group comparison with 5% type I error using a t-test provides power greater than 80%.

6.3 Evaluation for Safety

The safety analyses will include all randomised subjects who received study drug including placebo, with subjects grouped according to the treatment received. Safety parameters to be evaluated include AEs (including deaths, SAEs, discontinuations due to AEs, and the incidence and severity of AEs), clinical laboratory tests, vital signs, and ECGs. All collected

AE data will be listed by study site and subject number. All AEs that occur on or after treatment on Day 1 will be summarised by system organ class and preferred term as well as toxicity grade (severity). Post procedural pain will be captured as AEs and summarised separately. In addition, all SAEs, including deaths, will be listed separately and summarised. SAEs caused by a protocol mandated intervention (e.g., discontinuation of medications) that occur between consent and first dose of study medication will be listed separately.
Laboratory data with values outside of the normal ranges will be identified. In addition, select laboratory data will be summarised by treatment group using descriptive summary statistics.
The absolute and percentage changes from baseline in vital sign parameters will be computed, and changes deemed clinically significant by the investigator will be noted. Appropriate descriptive summary statistics will be provided for all vital sign parameters.

6.4 Pharmacokinetic Analyses

In Part A, descriptive linezolid PK analyses will be performed enabling a choice of dosing regimen which is estimated to give approximately 8 days of exposure within the disc.
In Part B, descriptive linezolid PK analyses will be performed, for the dosing regimen selected in Part A, which is estimated to give approximately 8 days of exposure within the disc (on the active arm).
The concentrations of selected excipients in plasma may be determined and summarised using descriptive PK analyses using retained or spare samples after the linezolid assay has been completed.

6.5 Evaluation for Efficacy

Part A: The efficacy results will be listed and summarised.
Part B: The primary efficacy endpoint will be analysed using a mixed model for repeated measures (MMRM) and will adjust for baseline to test the difference in the two groups for the LBP NRS score change from baseline at 12 months.

For change from baseline for LBP NRS at 12 months, A 1-sided hypothesis test will be performed to test the primary hypothesis of superiority of PP353 versus placebo at 12 months, that the reduction of LBP NRS score at Month 12 relative to Baseline for PP353 is greater than the reduction of LBP NRS score at Month 12 relative to Baseline for placebo.
The null hypothesis is that PP353 is not superior to Placebo at 12 months, that the reduction of LBP NRS score at Month 12 relative to Baseline for PP353 is not greater than the

reduction of LBP NRS score at Month 12 relative to Baseline for placebo. A 2-sided hypothesis test will also be performed but this will be separate to the primary analysis as the study is powered on the basis of a 1-sided test.

The model will use all available scheduled post-baseline LBP NRS score data at all visits up to and including 12 months and will be incorporated as repeated measures within each subject.

Treatment group (PP353 or placebo), visit, baseline LBP NRS score and treatment group by visit interaction will be included as fixed effects. The multiple visits for each subject will be incorporated as repeated measures within each subject. An unstructured covariance matrix will be utilized unless the model does not converge, in which case alternative covariance structures will be investigated. Least squares means (LS means) for changes from baseline and 95% 1-sided confidence intervals (CI) for each treatment group will be presented. A 2-sided 95% CI will also be presented.

Missing data are not explicitly imputed in the primary MMRM analysis, although there is an underlying assumption that data are missing at random. All available scheduled post-baseline assessments are utilised and, via modelling of the within subject correlation structure, the derived treatment differences at Month 12 are adjusted to take into account missing data. A mixed effects model for repeated measures analysis (using fixed effects and an unstructured variance covariance matrix) is considered appropriate as the primary method of analysis as it has been shown to give sensible answers to on-treatment questions in a range of practical situations (Siddiqui *et al.*, 2009).

Further details on analyses of the primary efficacy endpoint using the FAS will be provided in the SAP.

The primary efficacy endpoint may also be analysed using Bayesian analysis with an informative prior for the placebo group, if appropriate.
The secondary efficacy variables will be listed, summarised, and where appropriate, analysed using statistical testing.
All other efficacy variables will be listed, summarised, and analysed (where appropriate) using statistical testing.

6.6 Interim Analyses

Interim Analysis – Part A:

A formal review of the safety and PK will be conducted by the SRC after all subjects have completed the PK sampling for Part A. This will be used to identify the dosing regimen and timings of data collection for Part B.

In addition to the review of the safety and PK data for Part B dosing regimen decision, the SRC will also review the utility of the contrast enhanced MRI of available images from all the subjects in Part A and available Part B screening MRIs to make recommendations on the scientific value for inclusion of the 6 month DCE- MRI scan in Part B of the study.

6.7 Early Read-out - Part B

In Part B, there may be up to two Sponsor-unblinded early read-outs to review summary results. It is likely that the first will be conducted after all subjects have completed the Month 6 visit and the second after all subjects have completed the Month 9 visit. This will not alter the conduct of the Persica 002 study. Analyses will be conducted to enable the Sponsor to plan for future studies and for confidential investor discussions important to the future of Persica Pharmaceuticals. Details of the early read-out analyses may be provided in a separate SAP.

7. Data Collection and Management

7.1 Data Quality Assurance

Data management of the study will be performed under the responsibility of the Sponsor by the CRO. Data entered manually will be collected via EDC through use of eCRFs. Sites will be responsible for data entry into the EDC system. In the event of discrepant data, the Sponsor will request data clarification from the sites, which the sites will resolve electronically in the EDC system.

The CRO in consultation with the Sponsor will produce an Integrated Data Quality Review Plan that describes the quality checking to be performed on the data. Central laboratory data will be sent directly to the database, using standard procedures to handle and process the electronic transfer of these data.

eCRFs and correction documentation will be maintained in the EDC system's audit trail. System backups for data stored by the Sponsor and records retention for the study data will be consistent with the CRO's standard procedures.

Diary entries by subjects will be captured directly in the EDC system via an e-diary or on paper and then transferred to the EDC system by the investigator staff.

All imaging data collected in the study will be quality controlled through a central system by an imaging CRO.

7.2 Electronic Case Report Forms

eCRFs are to be completed through use of a Sponsor-designated EDC system. Sites will receive training and have access to a manual for appropriate eCRF completion. eCRFs will be submitted electronically and should be handled in accordance with instructions from the Sponsor.

All eCRFs should be completed by designated, trained site staff. eCRFs should be reviewed and electronically signed and dated by the investigator or a designee.

At the end of the study, the investigator will receive subject data for his or her site in a readable format on a compact disc that must be kept with the study records. Acknowledgement of receipt of the compact disc is required.

7.3 Source Data Documentation

The EDC system is an electronic data capturing and information management system that will also serve as the data management system for this study. The system combines all aspects of source data capturing with process control and clinical study management. All clinical and laboratory data, except those which are paper based, will be collected in the EDC. The responsible study Monitor will check data at the monitoring visits to the clinical study site. The investigator will ensure that the data collected are accurate, complete, and legible. Data will be monitored within EDC by the study Monitor who has only reading rights. Any changes required following monitoring will be made by site personnel or the investigator and will be documented with a full audit trail within EDC.

At a minimum, source documentation must be available for the following: subject identification, eligibility, and study identification; date of informed consent, dates of visits, PK sampling times, results of safety and efficacy parameters as required by the protocol, record of all AEs, follow up of AEs, concomitant medication, drug administration injection worksheet, drug receipt/dispensing/return records, study drug administration information, laboratory printouts (if not available digitally), date of study completion, and reason for early discontinuation of study drug or withdrawal from the study, if applicable.

Source documents, including e-source, must be attributable, legible, contemporaneous, original, accurate and complete (ALCOAC). The requirements also apply to any changes made in source documents.

Source data may be directly captured from devices, transferred from third parties (e.g., laboratory data), or entered manually into the EDC system in use at the clinical centre. In

such case, the majority of the source data will only be available electronically. The remainder of the data, captured initially on paper, may be entered retrospectively into the EDC system. Following the ICH/GCP guidelines, direct access to (e-)source documentation (medical records) must be allowed.

7.4 Use of Computerised Systems

When clinical observations are entered directly into a study site's computerised medical record system (i.e., in lieu of original hardcopy records), the electronic record can serve as the source document if the system has been validated in accordance with health authority requirements pertaining to computerised systems used in clinical research. An acceptable computerised data collection system allows preservation of the original entry of data. If original data are modified, the system should maintain a viewable audit trail that shows the original data as well as the reason for the change, name of the person making the change, and date of the change.

7.5 Retention of Records

Records and documents pertaining to the conduct of this study and the distribution of IMP, including eCRFs, paper diaries, ICFs, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for

- at least 15 years after completion or discontinuation of the trial,
- or for at least two years after formal discontinuation of clinical development of the investigational product,
- or for the length of time required by relevant national or local health authorities, whichever is longer.

After that period of time, the documents may be destroyed, subject to local regulations. No records may be disposed of without the written approval of the Sponsor. Written notification should be provided to the Sponsor prior to transferring any records to another party or moving them to another location.

8. Ethical Considerations

8.1 Compliance with Laws and Regulations

This study will be conducted in full conformance with the ICH GCP and the principles of the Declaration of Helsinki, or the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study will comply with the requirements of the ICH E2A guideline (Clinical Safety Data Management: Definitions and Standards for Expedited Reporting).

8.2 Informed Consent

Each subject must give written consent according to local requirements after the nature of the study has been fully explained. The consent form must be signed before performance of any study-related activity. The clinical records for each subject must document the informed consent process and that written informed consent was obtained prior to participation in the study. The consent form that is used must be approved by both the Sponsor and by the reviewing IEC. The informed consent should be in accordance with principles that originated in the Declaration of Helsinki, current ICH and GCP guidelines, applicable regulatory requirements, and Sponsor policy.

A copy of each signed Consent Form must be provided to the subject or the subject's legally authorised representative. All signed and dated Consent Forms must remain in each subject's medical records or in the site file and must be available for verification by study Monitors at any time.

8.3 Ethics Committee

An IEC should safeguard the rights, safety, and well-being of all study subjects.

Before the start of the study, the investigator (or Sponsor where required) will provide the IEC with current and complete documents required for review and approval according to the Standard Operating Procedures of the IEC including but not limited to:

- Final protocol and, if applicable, amendments
- Sponsor-approved ICF (and any updates) and any other written materials to be provided to the subjects;
- Sponsor-approved subject recruiting materials
- Investigator Brochure (or equivalent information) and addenda
- Available safety information
- Information on compensation for study-related injuries or payment to subjects for participation in the study, if applicable
- Investigator's current curriculum vitae or other documentation evidencing qualifications (unless not required, as documented by IEC)
- Information regarding funding, name of the Sponsor, institutional affiliations, other potential conflicts of interest, and incentives for subjects;
- Any other documents that the IEC may require to fulfil its obligation

This study may only commence at the site after the IEC has written approval of the final protocol and amendments (if any), the ICF(s) and updates (if any), applicable recruiting materials, and any other written information to be provided to the subjects, and the Sponsor has received a copy of this approval. This approval letter must be dated and must clearly identify the IEC and the documents being approved.

During the study, the investigator (or Sponsor where required) will send the following documents and updates to the IEC for their review and approval, where appropriate:

- Protocol amendments
- Revision(s) to ICF and any other written materials to be provided to subjects;
- if applicable, new or revised subject recruiting materials approved by the Sponsor;
- Revisions to compensation for study-related injuries or payment to subjects for participation in the study, if applicable
- Investigator's Brochure addenda or new edition(s)
- Summaries of the status of the study at intervals stipulated in guidelines of the IEC (at least annually)
- Reports of AEs that are serious, unlisted, and associated with the investigational drug
- New information that may adversely affect the safety of the subjects or the conduct of the study
- Deviations from or changes to the protocol to eliminate immediate hazards to the subjects;
- Report of deaths of subjects under the investigator's care
- Notification/submission of amendment if a new investigator is responsible for the study at the site
- Development Safety Update Report, Short Term Study Specific Safety Summary and Line Listings, where applicable
- Any other requirements of the IEC

For all protocol amendments (excluding the ones that are purely administrative, with no consequences for subjects, data or study conduct), the amendment and applicable ICF revisions must be submitted promptly to the IEC for review and approval before implementation of the change(s), except when necessary to eliminate immediate hazard to the study subjects. If a deviation from, or a change to the protocol was implemented to eliminate an immediate hazard to study subjects, then the implemented deviation or change, the reasons for it, and, if appropriate, the protocol amendment should be submitted to the IEC as soon as possible.

The investigator (or Sponsor where required) will notify the IEC about the end of study (defined in Section 3.2) within 90 days after the end of the study.

8.4 Confidentiality

The Sponsor maintains confidentiality standards by coding each subject enrolled in the study through assignment of a unique subject identification number. This means that subject names are not included in data sets that are transmitted to any Sponsor location.

Subject medical information obtained by this study is confidential and may be disclosed to third parties only as permitted by the Informed Consent Form (or separate authorisation for use and disclosure of personal health information) signed by the subject, unless permitted or required by law.

Medical information may be given to a subject's personal physician or other appropriate medical personnel responsible for the subject's welfare, for treatment purposes.

Data generated by this study must be available for inspection upon request by representatives of the U.S. FDA and other national and local health authorities, Sponsor monitors, representatives, and collaborators, and the EC for each study site, as appropriate.

8.5 Financial Disclosure

Investigators will provide the Sponsor with sufficient, accurate financial information in accordance with local regulations to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate health authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after the end of the study.

9. Study Documentation, Monitoring, and Administration

9.1 Study Documentation

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented, including but not limited to the protocol, protocol amendments, Informed Consent Forms, and documentation of EC and governmental approval. In addition, at the end of the study, the investigator will receive the subject data, including an audit trail containing a complete record of all changes to data.

9.2 Protocol Deviations

The investigator should document and explain any protocol deviations. The investigator should promptly report any deviations that might have an impact on subject safety and data integrity to the Sponsor and to the EC in accordance with established EC policies and procedures.

9.3 Site Monitoring, Audits and Inspections

The Sponsor has ethical, legal, and scientific obligations to follow this trial in accordance with established research principles, ICH E6 GCP: Consolidated Guidance, and applicable

regulatory requirements and local laws. The Sponsor or an authorised representative will visit the trial site(s) during the trial, as well as communicate frequently via telephone, e mail, and written communications. During site visits inspection of study data, subjects' medical records, and eCRFs will be carried out to verify compliance with the study protocol and all applicable regulations. In addition, all investigators and trial site clinical personnel will undergo initial and ongoing training for this particular trial, and this training will be clearly documented.

The Sponsor's (or designee's) Quality Assurance Unit (or representative) may conduct trial site audits. Audits will include, but are not limited to, IMP supply, presence of required documents, the informed consent process, and comparison of eCRFs with source documents. The investigator(s) agrees to participate with audits.

Regulatory authorities may inspect the trial site(s) during or after the trial. The investigator(s) will cooperate with such inspections and will contact the Sponsor immediately if such an inspection occurs.

9.4 Publication of Data and Protection of Intellectual Property

Regardless of the outcome of a trial, the Sponsor is dedicated to openly providing information on the trial to healthcare professionals and to the public, both at scientific congresses and in peer-reviewed journals.

The investigator must agree to submit all manuscripts or abstracts to the Sponsor prior to submission for publication or presentation. This allows the Sponsor to protect proprietary information and to provide comments based on information from other studies that may not yet be available to the investigator.

In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicentre trials only in their entirety and not as individual centre data. In this case, a coordinating investigator will be designated by the Sponsor.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements. Any formal publication of the study in which contribution of Sponsor personnel exceeded that of conventional monitoring will be considered as a joint publication by the investigator and the appropriate Sponsor personnel.

Any inventions and resulting patents, improvements, and/or know-how originating from the use of data from this study will become and remain the exclusive and unburdened property of the Sponsor, except where agreed otherwise.

9.5 Protocol Amendments

Any protocol amendments will be prepared by the Sponsor. Protocol amendments will be submitted to the EC and to regulatory authorities in accordance with local regulatory requirements.

Approval must be obtained from the EC and regulatory authorities (as locally required) before implementation of any changes, except for changes necessary to eliminate an immediate hazard to subjects or changes that involve logistical or administrative aspects only (e.g., change in medical monitor or contact information).

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Appendix A: Schedule of Activities

Schedule of Activities - Part A (Single Injection)

Apart from Screening, all MRI scans, Day 1 pre-dose, Dose 1/Dose 2 and Month 12/End of study visits, subjects do not need to attend the hospital for scheduled visits providing all assessments can be performed and the subject is seen by a qualified health professional e.g., a study Doctor or Nurse (not a phlebotomist).

	Schedule of Activities - Part A (single injection)																	
		Intervention Period [Days][1]							**Follow up**									**Notes**
Procedure	**Screening Visit [2]**	**V1 In-patient**			**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**TC[3]**	**V9**	**TC[3]**	**V 10**	**TC[3]**	**V11 ET/ EoS**	
	Day -28 to day -1	Day 1 – pre-dose [4]	Day 1 : post dose	Day 2	Day 5	Day 7	Day 9	Day 11	Month 1 (Day 30)	Month 2 (Day 60)	Month 3 (Day 90)	Months 4 & 5 (Day 120,150)	Month 6 (Day 180)	Month 7 & 8 (Day 210, 240)	Month 9 (Day 270)	Month 10 & 11 (Day 300, 330)	Month 12 (Day 365)	
Permitted visit window (days)		**-1**	**0**	**0**	**0**	**0**	**0**	**0**	**±3**	**±3**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	
Inpatient/overnight stay		X[5]																Subject to stay overnight after the procedure and to remain recumbent for 8 h post dose
Informed consent	X																	If required, consent may be obtained before the start of the 28 day screening window
Documented Inclusion & exclusion criteria check	X	X																
Demography	X																	
Pain Catastrophizing Scale	X																	
Full physical exam	X																	
Abbreviated Physical exam		X			X	X	X	X	X	X	(X)		(X)		(X)		X	- (X) Abbreviated Physical Exams are to be performed at scheduled visits if clinically indicated.
Medical history (includes substance usage)	X	X																

	Schedule of Activities - Part A (single injection)																	
		Intervention Period [Days][1]							**Follow up**									**Notes**
Procedure	**Screening Visit [2]**	**V1 In-patient**			**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**TC[3]**	**V9**	**TC[3]**	**V 10**	**TC[3]**	**V11 ET/ EoS**	
	Day -28 to day -1	Day 1 – pre-dose [4]	Day 1 : post dose	Day 2	Day 5	Day 7	Day 9	Day 11	Month 1 (Day 30)	Month 2 (Day 60)	Month 3 (Day 90)	Months 4 & 5 (Day 120,150)	Month 6 (Day 180)	Month 7 & 8 (Day 210, 240)	Month 9 (Day 270)	Month 10 & 11 (Day 300, 330)	Month 12 (Day 365)	
Permitted visit window (days)		**-1**	**0**	**0**	**0**	**0**	**0**	**0**	**±3**	**±3**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	
Pregnancy test	X	X[6]							X				X[6]				X[6]	Negative urine pregnancy test required pre-dose and before each MRI (unless permanently surgically sterile)
HBsAg, HCV and HIV screening	X																	
Laboratory assessments[7]	X	X		X		X		X	X	X	(X)		(X)		(X)		X	- Haematology, biochemistry and urinalysis - (X) Laboratory assessments are to be performed at scheduled visits if clinically indicated.
12-lead ECG	X		X[8]														X	Screening, 12 h ± 2 h post dose on Day 1 and at Month 12
Vital signs: BP, HR, Temp and RR	X	X	X[9]	X	X	X	X	X	X	X	(X)		(X)		(X)		X	- (X) Vital signs are to be performed at scheduled visits if clinically indicated.
Height and weight	X	X															X	Weight only at Day 1 Pre-dose

	Schedule of Activities - Part A (single injection)																	
		Intervention Period [Days][1]							**Follow up**									**Notes**
Procedure	**Screening Visit [2]**	**V1 In-patient**			**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**TC[3]**	**V9**	**TC[3]**	**V 10**	**TC[3]**	**V11 ET/ EoS**	
	Day -28 to day -1	Day 1 – pre-dose [4]	Day 1 : post dose	Day 2	Day 5	Day 7	Day 9	Day 11	Month 1 (Day 30)	Month 2 (Day 60)	Month 3 (Day 90)	Months 4 & 5 (Day 120,150)	Month 6 (Day 180)	Month 7 & 8 (Day 210, 240)	Month 9 (Day 270)	Month 10 & 11 (Day 300, 330)	Month 12 (Day 365)	
Permitted visit window (days)		**-1**	**0**	**0**	**0**	**0**	**0**	**0**	**±3**	**±3**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	
MRI & DCE-MRI scan	X[10]												X				X	Screening scan to be performed between Day -28 and Day -7 after other screening tests confirm subject eligibility. Central imaging confirmation of eligibility required prior to randomisation. Negative urine pregnancy test required for all women prior to each MRI (unless permanently surgically sterile).
Randomisation		X																If required randomisation can be done on the working day prior to first dose.
Intervertebral injection under image guidance		X[5]																Fluoroscopy imaging to be captured and transmitted as per Procedure Manual and Imaging Manual
Assess injection site reaction			X[11]															
PK samples[12]		X	X	X	X	X	X	X										See PK sampling timepoints table
RMDQ-23 + additional questions	X	X							X	X	X		X		X		X	
Oswestry Disability Questionnaire		X									X		X				X	

	Schedule of Activities - Part A (single injection)																	
		Intervention Period [Days][1]							**Follow up**									**Notes**
Procedure	**Screening Visit [2]**	**V1 In-patient**			**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**TC[3]**	**V9**	**TC[3]**	**V 10**	**TC[3]**	**V11 ET/ EoS**	
	Day -28 to day -1	Day 1 – pre-dose [4]	Day 1 : post dose	Day 2	Day 5	Day 7	Day 9	Day 11	Month 1 (Day 30)	Month 2 (Day 60)	Month 3 (Day 90)	Months 4 & 5 (Day 120,150)	Month 6 (Day 180)	Month 7 & 8 (Day 210, 240)	Month 9 (Day 270)	Month 10 & 11 (Day 300, 330)	Month 12 (Day 365)	
Permitted visit window (days)		**-1**	**0**	**0**	**0**	**0**	**0**	**0**	**±3**	**±3**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	**±7**	
LBP and leg pain Numeric rating scales[13] (14-day recall, Appx. B)	X	X			X				X	X	X		X		X		X	Assessed at site at screening. Recorded by subject in diary from V1
Hours with LBP during the last 4 weeks	X	X							X	X	X		X		X		X	Assessed at site at screening. Recorded by subject in diary from V1
Subject Global perceived effect									X	X	X		X		X		X	Recorded by subject in diary
Subject reported LBP relieving medication use (subject diary)	X[14] *Training only*	X[15]							X[15]	X[15]	X[15]		X[15]		X[15]		X[15]	At screening subject will be trained on diary completion to record LBP-relieving medication use. Diary completion by the subject will commence on Day -7
AE Collection[16]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	From signing of ICF to end of study
Concomitant medication and procedures review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Report history of treatments for CLBP for a period of at least 1 year, and other treatments for a period of at least 3 months, prior to the screening visit

[1] Days /times of assessments might change for Subjects 4, 5 and 6, following review of PK data from the first 3 subjects in Part A. PK samples up to Day 11 will not exceed 14 samples. Change to the days/times of assessment and PK sampling times will not constitute a protocol amendment.

[2] Screening visit can be performed up to 28 days before Day 1. Where a washout period is required for any contraindicated or prohibited medications, consent may be obtained and the washout period may commence before the start of the 28 day screening window. If a subject has not had a standard of care MRI, a suitability MRI (without gadolinium), may be conducted after a subject has signed consent. A suitability MRI may be performed prior to the start of the 28-day screening window. All other screening procedures must be performed within the protocol specified window for screening.

[3] Telephone call to subject – confirm completion of e-Subject Questionnaire, assess AEs

[4] Day 1 pre-dose assessments can be performed in the 24 hours preceding the administration of the injection

[5] Subject to stay overnight after the procedure. Subject to remain recumbent for 8 hours post dose, undertake only modest activity levels that do not provoke pain for the first 14 days following injection, and only engage in activity within pain limits thereafter.

[6] Urine pregnancy test on Day 1 prior to dose administration and prior to each MRI. Other pregnancy tests to be evaluated using a serum sample.

[7] Includes haematology, biochemistry and urinalysis (see lab list - Section 4.5.3.3).

[8] Single 12-lead ECG to be recorded at screening, 12 h ± 2 h post dose on day 1 and at month 12. (See Section 4.5.3.4)

[9] Vitals signs: Pre-dose vital signs to be recorded in the subject medical records and the eCRF. Post-dose cardiovascular and neurological observations should be made every 30 mins for a minimum of 2 h or as per local guidelines. Any clinically significant adverse events noted must be reported in the subject records and the eCRF.

[10] Screening MRI scan to be performed after all other screening tests have confirmed subject eligibility. MRI can be conducted on a separate day within the screening period within the period Day -28 to Day -7. **Central MRI eligibility confirmation must be obtained prior to randomisation**. Follow directions for MRI acquisition and transmission provided in the Imaging Manual.

[11] Participants should be monitored after each injection at 30 min, 1 h, 8 h and immediately before discharge for any injection site reaction. Any injection site reaction observed should be reported as an AE according to the scale in Section 5.2.7

[12] See PK sampling timepoints table below

[13] Numeric rating scale to be completed for LBP and leg pain

[14] Subject to be trained on diary completion for recording LBP-relieving medication use during screening period. E-Diary completion by the subjects will commence on Day -7

[15] Subject reported analgesic use for LBP to be recorded daily in the e-diary for the 7 days preceding the study visit

[16] AEs to be reported from signing of ICF to end of study

Part A: PK Sampling timepoints for Subjects 1-3

The timepoints for PK sample collection may change for Subjects 4-6 and one extra visit may be necessary. The total number of PK samples in Part A, up to and including Day 11 will not exceed 14 samples.

Timepoint	**Day 1 (Pre-dose / post-dose)**	**Day 2**	**Day5**	**Day7**	**Day 9**	**Day 11**
Sampling Window	See below	See below	±3 h	±3 h	±3 h	±3 h
Pre-dose	Baseline (-30min)					
Post dose	30 min (±15 min)	24 h (±30 min)	96 h	144 h	192 h	240 h
	2 h (±30 min)	30 h (±3 h)				
	4 h (±30 min)					
	8 h (±30 min)					
	12 h (±30 min)					

Schedule of Activities - Part B (Two Injections)

The number of injections in Part B was determined by the PK observed in Part A of the study; two injections of up to 3 mL each will be administered on Day 1 and then Day 5 (±1 day).

Apart from Screening, all MRI scans, Day 1 pre-dose, Dose 1/Dose 2 and Month 12/End of study visits, subjects do not need to attend the hospital for scheduled visits providing all assessments can be performed and the subject is seen by a qualified health professional e.g., a study Doctor or Nurse (not a phlebotomist).

Procedure	Schedule of Activities - Part B (Two injections, administered on Day 1 and then Day 5 (±1 day)																		
		Intervention Period [Days]									Follow-up [Days]								Notes
	Screening Visit[17]	V1 Dose 1		V2	V3 Dose 2		V4	V5	V6	V7	TC[18]	V8	TC[18]	V9	TC[18]	V10	TC[18]	V11 ET/ EoS	
	Day -56 to Day -1	Day 1 - pre-dose[19]	Day 1 – Dose 1	Day 3	Dose 2 - Pre-dose[20]	Dose 2 – post dose (Day 5)	Dose 2 + 2 Days (e.g. Day 7)	Dose 2 + 4 Days (e.g. Day 9)	Dose 2 + 6 Days (e.g. Day 11)	Month 1 (Day 30)	Month 2 (Day 60)	Month 3 (Day 90)	Months 4 & 5 (Day 120 & 150)	Month 6 (Day 180)	Month 7 & 8 (Day 210 & 240)	Month 9 (Day 270)	Month 10 & 11 (Day 300 & 330)	Month 12 (Day 365)	
Permitted visit window (days)		0		*	±1		*	*	*	±3	±3	±7	±7	±7	±7	±7	±7	±7	Apart from visits 4, 5 and 6, all visit dates are determined from date of Day 1 Dose 1. * Refer to PK Schedule of Activities Table
Informed consent	X																		If required, consent may be obtained before the start of the 56-day screening window.
Documented incl. & excl. criteria check	X	X																	
Demography	X																		
Pain Catastrophizing Scale	X																		
Full physical exam	X																		
Abbreviated physical exam		X		*	**X**		(X)*	(X)	(X)*	X		(X)		(X)		(X)		X	(X) Abbreviated physical exams are to be performed at scheduled visits if clinically indicated. **X** Pre dose assessment may be performed up to **48 hrs** before Dose 2 *Only first 12 subjects in Part B will attend site for V2, V4, and V6. After the 12th patient, these visits are TC[18] visits
Medical history (includes substance usage)	X	X																	

	Schedule of Activities - Part B (Two injections, administered on Day 1 and then Day 5 (±1 day)																	
		Intervention Period [Days]								**Follow-up [Days]**								**Notes**
	Screening Visit[17]	V1 Dose 1		V2	V3 Dose 2		V4	V5	V6	V7	TC[18]	V8	TC[18]	V9	TC[18]	V10	TC[18]	V11 ET/ EoS	
Procedure	Day -56 to Day -1	Day 1 - pre-dose[19]	Day 1 – Dose 1	Day 3	Dose 2 - Pre-dose[20]	Dose 2 – post dose (Day 5)	Dose 2 + 2 Days (e.g. Day 7)	Dose 2 + 4 Days (e.g. Day 9)	Dose 2 + 6 Days (e.g. Day 11)	Month 1 (Day 30)	Month 2 (Day 60)	Month 3 (Day 90)	Months 4 & 5 (Day 120 & 150)	Month 6 (Day 180)	Month 7 & 8 (Day 210 & 240)	Month 9 (Day 270)	Month 10 & 11 (Day 300 & 330)	Month 12 (Day 365)	
Permitted visit window (days)		0		*	±1		*	*	*	±3	±3	±7	±7	±7	±7	±7	±7	±7	Apart from visits 4, 5 and 6, all visit dates are determined from date of Day 1 Dose 1. * Refer to PK Schedule of Activities Table
Pregnancy test	X **S**	X **U**			**X** **U**					X **S**				X **U**				X **U**	- **S**erum pregnancy test on all women at screening and all women (unless permanently surgically sterile) at Day 30. - **U**rine pregnancy test on all women (unless permanently surgically sterile) prior to each dose administration and before each MRI. - **X** Pre dose assessment may be performed up to **48 hrs** before Dose 2
HBsAg, HCV and HIV screening	X																		
Laboratory assessments	X	X		*	**X**				X*	X		(X)		(X)		(X)		X	- Haematology, biochemistry and urinalysis (see lab list - Section 4.5.3.3). - (X) Laboratory assessments are to be performed at scheduled visits if clinically indicated. - **X** Pre dose assessment may be performed up to **48 hrs** before Dose 2 - *Only first 12 subjects in Part B will attend site for V2, V4 and V6. After the 12th patient, these visits are TC[18] visits

	Schedule of Activities - Part B (Two injections, administered on Day 1 and then Day 5 (±1 day)																	
		Intervention Period [Days]								**Follow-up [Days]**								**Notes**
	Screening Visit[17]	V1 Dose 1		V2	V3 Dose 2		V4	V5	V6	V7	TC[18]	V8	TC[18]	V9	TC[18]	V10	TC[18]	V11 ET/ EoS	
Procedure	Day -56 to Day -1	Day 1 - pre-dose[19]	Day 1 – Dose 1	Day 3	Dose 2 - Pre-dose[20]	Dose 2 – post dose (Day 5)	Dose 2 + 2 Days (e.g. Day 7)	Dose 2 + 4 Days (e.g. Day 9)	Dose 2 + 6 Days (e.g. Day 11)	Month 1 (Day 30)	Month 2 (Day 60)	Month 3 (Day 90)	Months 4 & 5 (Day 120 & 150)	Month 6 (Day 180)	Month 7 & 8 (Day 210 & 240)	Month 9 (Day 270)	Month 10 & 11 (Day 300 & 330)	Month 12 (Day 365)	
Permitted visit window (days)		0		*	±1		*	*	*	±3	±3	±7	±7	±7	±7	±7	±7	±7	Apart from visits 4, 5 and 6, all visit dates are determined from date of Day 1 Dose 1. * Refer to PK Schedule of Activities Table
12-lead ECG	X		**X**			**X**												X	- 12-lead ECG to be obtained in duplicate (see Section 4.5.3.4) - **X** Day 1 & Dose 2 ECG's 8 h ± 2 h **post-dose** - Post dose assessments are calculated from the start time of the injection as documented on "Injection Worksheet Persica Study 002 **Form 2"**.

	Schedule of Activities - Part B (Two injections, administered on Day 1 and then Day 5 (±1 day)																	
		Intervention Period [Days]									**Follow-up [Days]**							**Notes**
	Screening Visit[17]	V1 Dose 1		V2	V3 Dose 2		V4	V5	V6	V7	TC[18]	V8	TC[18]	V9	TC[18]	V10	TC[18]	V11 ET/ EoS	
Procedure	Day -56 to Day -1	Day 1 - pre-dose[19]	Day 1 – Dose 1	Day 3	Dose 2 - Pre-dose[20]	Dose 2 – post dose (Day 5)	Dose 2 + 2 Days (e.g. Day 7)	Dose 2 + 4 Days (e.g. Day 9)	Dose 2 + 6 Days (e.g. Day 11)	Month 1 (Day 30)	Month 2 (Day 60)	Month 3 (Day 90)	Months 4 & 5 (Day 120 & 150)	Month 6 (Day 180)	Month 7 & 8 (Day 210 & 240)	Month 9 (Day 270)	Month 10 & 11 (Day 300 & 330)	Month 12 (Day 365)	
Permitted visit window (days)		0		*	±1		*	*	*	±3	±3	±7	±7	±7	±7	±7	±7	±7	Apart from visits 4, 5 and 6, all visit dates are determined from date of Day 1 Dose 1. * Refer to PK Schedule of Activities Table
Vital signs (BP, HR, Temp and RR)	X	**<X>**	**X**	(X)*	**<X>**	**X**	(X)*	(X)	(X)*	X		(X)		(X)		(X)		X	**- <X>** Day 1 and Dose 2 **pre-dose VS assessment must be performed on the same day as Dosing** - **X** Day 1 and Dose 2 **post-dose** cardiovascular and neurological observations should be made every 30 min for a minimum of 2 h or as per local guidelines. Any clinically significant adverse events noted must be reported in the subject records and the eCRF. - Post dose assessments are calculated from the start time of the injection as documented on "Injection Worksheet Persica Study 002 **Form 2"**. - (X) Vital signs are to be performed at scheduled visits if clinically indicated. - *Only first 12 subjects in Part B will attend site for V2, V4, and V6. After the 12th patient, these visits are TC[18] visits
Height & weight	X	**X**																X	**X** Weight only at Day 1 Pre-dose

Procedure	Schedule of Activities - Part B (Two injections, administered on Day 1 and then Day 5 (±1 day)																	Notes
		Intervention Period [Days]									Follow-up [Days]							
	Screening Visit[17]	V1 Dose 1		V2	V3 Dose 2		V4	V5	V6	V7	TC[18]	V8	TC[18]	V9	TC[18]	V10	TC[18]	V11 ET/ EoS	
	Day -56 to Day -1	Day 1 - pre-dose[19]	Day 1 – Dose 1	Day 3	Dose 2 - Pre-dose[20]	Dose 2 – post dose (Day 5)	Dose 2 + 2 Days (e.g. Day 7)	Dose 2 + 4 Days (e.g. Day 9)	Dose 2 + 6 Days (e.g. Day 11)	Month 1 (Day 30)	Month 2 (Day 60)	Month 3 (Day 90)	Months 4 & 5 (Day 120 & 150)	Month 6 (Day 180)	Month 7 & 8 (Day 210 & 240)	Month 9 (Day 270)	Month 10 & 11 (Day 300 & 330)	Month 12 (Day 365)	
Permitted visit window (days)		0		*	±1		*	*	*	±3	±3	±7	±7	±7	±7	±7	±7	±7	Apart from visits 4, 5 and 6, all visit dates are determined from date of Day 1 Dose 1. * Refer to PK Schedule of Activities Table
MRI & DCE-MRI scan - DCE-MRI sequences should be done on all subjects where possible. In cases where the DCE-MRI procedure cannot be conducted by the site or the subject is unable to tolerate the procedure, the DCE-MRI sequence can be omitted. However, all other sequences should be performed. If the screening DCE-MRI has not been conducted, then DCE-MRI should not be performed at the 6 or 12 month visit.	X													X				X	- Screening scan to be performed between Day -56 (and approximately Day -7) after all other screening tests confirm subject eligibility. **Central imaging confirmation of eligibility required prior to randomisation.** - Negative urine pregnancy test required for all women prior to each MRI (unless permanently surgically sterile). - Follow directions for MRI acquisition in the imaging manual and image acquisition manual.
Randomisation (Day 1 Dose 1) + additional IRT transactions		**X**			**X**				(X)										- Subjects can be randomised only after all entry criteria have been met. - **X** If required, randomisation (Day 1 Dose 1) and additional dispensing (Dose 2) can be done 72 hours before each dosing. - (X) Register the end of treatment in IRT.

Procedure	Schedule of Activities - Part B (Two injections, administered on Day 1 and then Day 5 (±1 day)																	
		Intervention Period [Days]								Follow-up [Days]								Notes
	Screening Visit[17]	V1 Dose 1		V2	V3 Dose 2		V4	V5	V6	V7	TC[18]	V8	TC[18]	V9	TC[18]	V10	TC[18]	V11 ET/ EoS	
	Day -56 to Day -1	Day 1 - pre-dose[19]	Day 1 – Dose 1	Day 3	Dose 2 - Pre-dose[20]	Dose 2 – post dose (Day 5)	Dose 2 + 2 Days (e.g. Day 7)	Dose 2 + 4 Days (e.g. Day 9)	Dose 2 + 6 Days (e.g. Day 11)	Month 1 (Day 30)	Month 2 (Day 60)	Month 3 (Day 90)	Months 4 & 5 (Day 120 & 150)	Month 6 (Day 180)	Month 7 & 8 (Day 210 & 240)	Month 9 (Day 270)	Month 10 & 11 (Day 300 & 330)	Month 12 (Day 365)	
Permitted visit window (days)		0		*	±1		*	*	*	±3	±3	±7	±7	±7	±7	±7	±7	±7	Apart from visits 4, 5 and 6, all visit dates are determined from date of Day 1 Dose 1. * Refer to PK Schedule of Activities Table
Intervertebral disc injection under image guidance		X			X														- Negative urine pregnancy test required for all women prior to each injection (unless permanently surgically sterile). - Subjects in Part B must have limited activity for at least 8 hrs after each injection in order to maximise the opportunity for the study medication to remain within the intervertebral disc space; they should remain lying down on bed rest, e.g. recumbent, semi-recumbent or on their side, for the first 4 hours and then have limited activity for the next 4 hours after each injection as described in the Procedure Manual. - Imaging of each dose administration to be captured and transmitted as per Procedure Manual, Image Acquisition Manual and Imaging Manual.

Procedure	Schedule of Activities - Part B (Two injections, administered on Day 1 and then Day 5 (±1 day)																	Notes
		Intervention Period [Days]									Follow-up [Days]							
	Screening Visit[17]	V1 Dose 1		V2	V3 Dose 2		V4	V5	V6	V7	TC[18]	V8	TC[18]	V9	TC[18]	V10	TC[18]	V11 ET/ EoS	
	Day -56 to Day -1	Day 1 - pre-dose[19]	Day 1 – Dose 1	Day 3	Dose 2 - Pre-dose[20]	Dose 2 – post dose (Day 5)	Dose 2 + 2 Days (e.g. Day 7)	Dose 2 + 4 Days (e.g. Day 9)	Dose 2 + 6 Days (e.g. Day 11)	Month 1 (Day 30)	Month 2 (Day 60)	Month 3 (Day 90)	Months 4 & 5 (Day 120 & 150)	Month 6 (Day 180)	Month 7 & 8 (Day 210 & 240)	Month 9 (Day 270)	Month 10 & 11 (Day 300 & 330)	Month 12 (Day 365)	
Permitted visit window (days)		0		*	±1		*	*	*	±3	±3	±7	±7	±7	±7	±7	±7	±7	Apart from visits 4, 5 and 6, all visit dates are determined from date of Day 1 Dose 1. * Refer to PK Schedule of Activities Table
Assess injection site reaction			X			X													- Post dose assessments are calculated from the start time of the injection as documented on "Injection Worksheet Persica Study 002 **Form 2"**. - Should be monitored after each injection at 30 min, 1h, and 8h for any injection site reaction. - If subject stays in hospital >12 h after an injection procedure, then an assessment "immediately prior to discharge" should be conducted. - Any injection site reaction observed should be reported as an adverse event according to the scale in Section 5.2.7.
PK samples		X	X	X*	X	X	X*	X	X*										Refer to PK Schedule of Activities table below. * Only first 12 subjects in Part B will have full PK testing. After the 12th patient, V2, V4, and V6 are TC[18] visits
RMDQ -23 + additional questions	X	X								X		X		X		X		X	Completed on paper.
Oswestry Disability Questionnaire		X										X		X				X	Completed on paper.

Procedure	Schedule of Activities - Part B (Two injections, administered on Day 1 and then Day 5 (±1 day)																	
		Intervention Period [Days]								Follow-up [Days]								Notes
	Screening Visit[17]	V1 Dose 1		V2	V3 Dose 2		V4	V5	V6	V7	TC[18]	V8	TC[18]	V9	TC[18]	V10	TC[18]	V11 ET/ EoS	
	Day -56 to Day -1	Day 1 - pre-dose[19]	Day 1 – Dose 1	Day 3	Dose 2 - Pre-dose[20]	Dose 2 – post dose (Day 5)	Dose 2 + 2 Days (e.g. Day 7)	Dose 2 + 4 Days (e.g. Day 9)	Dose 2 + 6 Days (e.g. Day 11)	Month 1 (Day 30)	Month 2 (Day 60)	Month 3 (Day 90)	Months 4 & 5 (Day 120 & 150)	Month 6 (Day 180)	Month 7 & 8 (Day 210 & 240)	Month 9 (Day 270)	Month 10 & 11 (Day 300 & 330)	Month 12 (Day 365)	
Permitted visit window (days)		0		*	±1		*	*	*	±3	±3	±7	±7	±7	±7	±7	±7	±7	Apart from visits 4, 5 and 6, all visit dates are determined from date of Day 1 Dose 1. * Refer to PK Schedule of Activities Table
LBP and leg pain Numeric rating scales (14-day recall, Appx. B)[21]	X	**X**								**X**		**X**		**X**		**X**		**X**	- X Assessed on paper at screening. - **X** either on paper or e-diary (country dependent).
Hours with LBP during the last 4 weeks[21]	X	X								X		X		X		X		X	- Assessed on paper at screening. - Then either on paper or e-diary (country dependent).
Subject Global perceived effect[21]										X		X		X		X		X	Assessed on paper or e-diary (country dependent).
7 Day average LBP NRS daily score[21]		X								X		X		X		X		X	- Recorded by subject each day for 7 days prior to scheduled visit date. - Assessed on paper or e-diary (country dependent).
7 Day Subject reported LBP relieving medication use (subject diary) [21]	**X**	X								X		X		X		X		X	- **X At screening subject will be trained on diary completion.** - Diary completion starts 7 days before Day 1 pre-dose, then recorded by subject each day for 7 days prior to scheduled visit date. - Assessed on paper or e-diary (country dependent).

	Schedule of Activities - Part B (Two injections, administered on Day 1 and then Day 5 (±1 day)																	
		Intervention Period [Days]									**Follow-up [Days]**							**Notes**
	Screening Visit[17]	V1 Dose 1		V2	V3 Dose 2		V4	V5	V6	V7	TC[18]	V8	TC[18]	V9	TC[18]	V10	TC[18]	V11 ET/ EoS	
Procedure	Day -56 to Day -1	Day 1 - pre-dose[19]	Day 1 – Dose 1	Day 3	Dose 2 - Pre-dose[20]	Dose 2 – post dose (Day 5)	Dose 2 + 2 Days (e.g. Day 7)	Dose 2 + 4 Days (e.g. Day 9)	Dose 2 + 6 Days (e.g. Day 11)	Month 1 (Day 30)	Month 2 (Day 60)	Month 3 (Day 90)	Months 4 & 5 (Day 120 & 150)	Month 6 (Day 180)	Month 7 & 8 (Day 210 & 240)	Month 9 (Day 270)	Month 10 & 11 (Day 300 & 330)	Month 12 (Day 365)	
Permitted visit window (days)		0		*	±1		*	*	*	±3	±3	±7	±7	±7	±7	±7	±7	±7	Apart from visits 4, 5 and 6, all visit dates are determined from date of Day 1 Dose 1. * Refer to PK Schedule of Activities Table
AE Collection	X	X	**X**	**X**	X	**X**	**X**	X	X	X	X	X	X	X	X	X	X	X	- AE s to be reported from signing of ICF to end of study. - **X** Signs and symptoms of serotonergic syndrome (e.g., agitation, increase BP, increase HR) should be looked for after each injection and the first visit post injection
Con med and procedures review	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	Report history of treatments for CLBP for a period of at least 1 year, and other treatments for a period of at least 3 months, prior to the screening visit

[17] Screening visit can be performed up to 56 days before Day 1. If a subject has not had a standard of care MRI, a suitability MRI (without gadolinium), may be conducted after a subject has signed consent. Sites may administer a suitability assessment of the RMDQ-23 questionnaire and Low Back Pain Numeric Rating Scale/Leg Pain Numeric Rating Scale to avoid sending ineligible subjects for suitability MRIs. These suitability assessments are not databased. A suitability MRI suitability RMDQ-23 and suitability Low Back Pain Numeric Rating Scale/Leg Pain Numeric Rating Scale may be performed prior to the start of the 56-day screening window. All other screening procedures (including screening RMDQ-23 and suitability Low Back Pain Numeric Rating Scale/Leg Pain Numeric Rating Scale) must be performed within the protocol specified window for screening (unless the Sponsor has agreed an extension).

[18] Telephone call to subject – assess AE's and Concomitant medications.

[19] Day 1 pre-dose assessments can be performed in the 72 hours preceding the administration of the injection, **except for Vital Sign assessments which must be performed on the day of dosing.**

[20] Dose 2 pre-dose assessments can be performed in the 48 hours preceding the administration of the injection, **except for Vital Sign assessments which must be performed on the day of dosing.**

[21] The e-diary has a 96-hour window for completion. Only missing, part-completed or inaccurate data will be reported as protocol deviations.

Dosing and PK Schedule of Activities - Part B (Two Injections)

The number of injections in Part B was determined by the PK observed in Part A of the study; two injections of up to 3 mL each will be administered on Day 1 and then Day 5 (±1 day).

Total of 2 doses, Day 1 and Day 5 – up to 16 PK blood samples*:

Timepoint	**DOSE 1 Day 1 Pre-dose & post-dose**	**Day 3***	**DOSE 2 (Day 5)**	**Dose 2 + 2 Days* (e.g. Day 7)**	**Dose 2 + 4 Days (e.g. Day 9)**	**Dose 2 + 6 Days* (e.g. Day 11)**
Sampling window	See below	-12 h / +24 h	± 1 Day All subsequent PK sampling days are calculated from the actual date of Dose 2	-12 h / +24 h	±24 h	±24 h
Pre-dose	Baseline (-24 hrs)		Predose (-120 min)			
Post dose**	1 h (± 15 min)	48 h	1 h (± 15 min)	48 h	96 h	144 h
	2 h (± 30 min)		2 h (± 30 min)			
	4 h (± 30 min)		4 h (± 30 min)			
	6 h (± 30 min)		6 h (± 30 min)			
	8 h (± 30 min)		8 h (± 30 min)			

***** Only first 12 subjects in Part B will have full PK testing. For all subsequent subjects, visits Day 3, Dose 2 + 2 Days and Dose 2 + 6 Days are TC^{18} visits, and PK samples will no longer be collected at these timepoints.

** All Post dose assessments are calculated from the start time of the injection as documented on "Injection Worksheet Persica Study 002 **Form 2"**.

Appendix F: Protocol Amendment(s)/Administrative Change(s)

Please refer to separate document(s) containing the summary of changes for each protocol amendment.