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RESEARCH ARTICLE

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# Preclinical development and characterisation of PP353, a formulation of linezolid for intradiscal administration

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## Abstract

**Introduction:** Bacterial infection of the intervertebral disc can lead to vertebral endplate edema known as Modic changes, with associated chronic low back pain. Oral antimicrobial therapy has shown efficacy but relies on prolonged dosing and may not be optimal in terms of patient outcome, side effects, or antibiotic stewardship. There is no antibiotic formulation approved for intradiscal administration. Here, we describe the development and preclinical characterization of a formulation of linezolid, a suspension of 50 mg/mL micronized powder, for intradiscal administration.

**Methods:** Micronization, particle size analysis, Franz cell diffusion assays, ex vivo bioassay, and estimates of gelling temperature were used to optimize the composition and properties of the formulation. Performance of the formulation was assessed using sheep to characterize the pharmacokinetics and a model of intradiscal infection was developed to demonstrate efficacy. Suitability for human administration was demonstrated in a Good Laboratory Practice (GLP) local tolerance study.

**Results:** Micronized linezolid, formulated as a powder suspension using a vehicle containing poloxamer 407 and iohexol, provided a temperature-dependent radio-opaque gel that was suitable for image-guided percutaneous intradiscal administration. Efficacy in a sheep model of intradiscal *Staphylococcus aureus* infection was demonstrated. The formulation provides a high level of sheep disc tissue exposure, with $C_{max}$ of 6500 μg/g and limited systemic exposure, with a plasma $C_{max}$ of 0.04 μg/mL per 0.1 mL dose (5 mg of linezolid). Deconvolution of plasma linezolid pharmacokinetics

correlated with linezolid remaining in the disc over time. Observations from a GLP local tolerance study with the linezolid formulation were of a minor nature and related to the intradiscal administration procedure.

**Conclusions:** Linezolid can be formulated for image-guided percutaneous intradiscal administration. The formulation is now in a Phase 1b clinical trial to evaluate safety, pharmacokinetics, and efficacy in patients with CLBP and suspected bacterial infection.

**KEYWORDS**
intradiscal, linezolid, Modic, pharmacokinetics, vertebrogenic back pain

## 1 | INTRODUCTION

Chronic bacterial infection of the vertebral disc has been associated with Modic changes type 1 (MC1) in adjacent vertebrae and MC1 has been associated with chronic low back pain (CLBP).[1,2] Treatments for CLBP with MC1 patients are largely ineffective, including conservative therapies,[3] medication[4] spinal injections,[5] and spinal fusions.[6] The chronic use of strong analgesic medication including opioids, neuropathic pain medication, and non-steroidal anti-inflammatory drugs is common.

The contribution of MC1 or Modic changes type 2 (MC2) on MRI to CLBP is becoming more widely appreciated and has led to the endorsement of a new diagnostic classification of CLBP patients with MC1 or MC2 changes as vertebrogenic low back pain by US learned societies with an International Classification of Diseases (10th Revision)-CM diagnostic code M54-51.[7–9]

Studies deploying quantitative microbiology demonstrate the presence of bacteria in almost half of disc tissue samples, and histology of disc tissue confirms that they are embedded as colonies in the disc matrix.[10–15] The most common bacterial species isolated from disc tissue samples is *Cutibacterium acnes* (formerly *Propionibacterium acnes*), found in about 36% of disc tissue samples, with Coagulase-negative *Staphylococcus*, *Corynebacterium*, *Peptostreptococcus*, Gram-positive bacilli, *Micrococcus*, *Neisseria*, and rarely (<2.5%) Gram-negative bacilli also being reported.[1,10–12,16–23] Disc degeneration and Modic changes have been induced in studies which introduce *C. acnes* derived from human disc tissue into rabbit and rat discs.[24–27] The bacterial burden in non-pyogenic chronic disc infection is low and may be below the limits of detection in some studies.[11,12,14,28,29]

Oral antibiotic treatment of patients with CLBP and MC1 has been evaluated in two similar RCTs.[30,31] Both studies administered amoxicillin or coamoxiclav for 100 days. Albert et al. found a substantial benefit from oral antibiotic treatment with significant reductions in both pain and disability over 12 months. Braten et al. confirmed a reduction in pain and disability with antibiotics, but with a reduced magnitude. A post hoc image-based subgroup analysis of the Braten et al. study identified subjects with large vertebral edema/Modic as antibiotic responders with substantial and significant reductions in pain and disability.[32] As bacteria are not isolated from all disc tissue samples it is possible that only a proportion of a patient population, those with bacterial infections of their discs, can respond to antibiotic therapy.

However, oral antimicrobial therapy for bacterial disc infection relies on prolonged dosing and may not be optimal in terms of patient outcome, side effects, or antibiotic stewardship. Ad hoc formulations of antibiotics used for intradiscal antibiotics have been described, for example, 2 g cefazolin or vancomycin twice a week, depending on bacterial infection for an average of 3.5 weeks, but no antibiotic formulation has been approved for intradiscal administration.[33]

Here, we describe the development and preclinical characterization of PP353, an antibiotic formulation for intradiscal administration to treat bacterial infection which may find use as an alternative or adjunct to current therapies.

The target product profile was defined as a generic antibiotic with activity against Gram-positive bacteria and a low frequency of resistance in clinical isolates that could be formulated for intradiscal administration to degenerate discs. The formulation had to be suitable for administration through long (178 mm) narrow bore (22G) spinal needles and had to be retained in degenerate, often fissured discs, so that it did not leak into adjacent tissues. Ideally, a radio-contrast agent would be included to enable image-guided administration. The antibiotic formulation should have sustained disc exposure so that few intradiscal administrations would provide an effective antibacterial exposure.

The process leading to the selection of linezolid is described in supplemental information (Section S1). Linezolid is an antibacterial drug that binds to the bacterial ribosomal RNA and prevents the formation of a functional 70S initiation complex, which is an essential component of the bacterial protein translation process. It has activity against aerobic Gram-positive bacteria and anaerobic microorganisms, including those resistant to methicillin and vancomycin.[34] Linezolid is approved for the treatment of pneumonia, complicated skin and soft-tissue infections caused by Gram-positive bacteria, and as a component of a treatment for tuberculosis. The standard dose of linezolid is 600 mg every 12 h by oral or intravenous administration. Linezolid is 100% bioavailable. Linezolid is a non-ionically charged oxazolidinone with a low molecular weight of 337.3 g/mol and low aqueous solubility of about 2 mg/mL.

Formal minimal inhibitory concentration (MIC) breakpoints for linezolid activity against susceptible strains of *C. acnes* are not

available because neither are indicated for acne treatment. Linezolid $MIC_{90}$ against *C. acnes* and *Staphylococcus aureus* is approximately 1 μg/mL.[35–40]

Formulation studies led to the development of PP353, consisting of a suspension of micronized linezolid crystal form II in a Poloxamer 407 thermosensitive gel containing iohexol. A large animal model, the sheep, was selected for pharmacokinetics, efficacy, and local tolerance studies as it enabled larger doses and use of similar syringes and needles as would be used in the clinic and represented a lower translational step to human characterization.

## 2 | METHODS

### 2.1 | Linezolid assays

Three linezolid assays were used. A HPLC assay for formulation analytical purposes, e.g., in vitro release studies, and an LC–MS/MS format for bioanalytical purposes, e.g., pharmacokinetics. The LC–MS/MS assay format included non-GLP and GLP versions. The details are provided in Section S5.

### 2.2 | Particle size distribution

The particle size distribution of the raw and air-jet milled linezolid was analyzed by laser diffraction (Helos Disperse, Sympatec GmbH, Germany). 5 mg of each sample was placed in a dry powder disperser (RODOS/M). A reference measurement was taken before running each sample for 5 seconds at 2% optical concentration. The results were obtained at a pressure of 3 bars using lenses, RI (0.18–0.35 μm) and R2 (0.25/0.45–87.5 μm). Data were collected using the HELOS sensor and analyzed using Windox5 software (Sympatec GmbH).

### 2.3 | Preparation of PP353

PP353 is a suspension of micronized linezolid crystal form II powder (PP353-A) in a vehicle for suspension composed of poloxamer 407 and iohexol plus pharmaceutical excipients (PP353-B). The preparation of PP353-A and PP353-B is described in detail in Sections S2–S4.

### 2.4 | Franz cell studies

The release of linezolid from the warmed poloxamer 407 hydrogel was evaluated using a Franz diffusion cell in which the donor compartment containing the test linezolid suspension is separated from the receptor compartment by a semipermeable 12–14 kDa Visking membrane. Diffusion of linezolid from donor to recipient compartment containing PBS was assessed by HPLC assay (Section S5).

### 2.5 | Sheep studies including intradiscal infection model, pharmacokinetics, and GLP local tolerance studies

The details of Sheep studies are described in the Section S6. All in vivo studies were carried out under a Home Office Project License (PPL) No. P59DC2C4F. All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986. The protocol was approved by the Royal Veterinary College Animal Welfare and Ethical Review Board. Disc injection was performed under general anesthesia that was induced with a combination of ketamine and midazolam intravenously and maintained with ~2% isoflurane vaporized in a mixture of oxygen and medical air. Perioperative analgesic (meloxicam) was administered to all animals. A 20G 88.9 mm spinal needle positioned directly on the edge of the intervertebral disc nucleus pulposus acted as a guide needle. The placement was confirmed by dorsal/ventral and lateral X-ray images. Administration to the nucleus pulposus used a 25G 119 mm spinal needle passed through the guide needle. Discs were infected by administration of 0.05 mL of $2 \times 10^4$ *S. aureus* ATCC29213 cells/mL.

### 2.6 | Intradiscal infection model with PP353-precursor formulation

Efficacy studies used a PP353-precursor formulation which contained 43.5 mg/mL micronized linezolid form II and 13% (w/w) poloxamer 407 and had a sol/gel transition at 34°C. To test the efficacy of the linezolid formulation, five sheep were split into three groups. Four discs in each animal were infected by injection of $10^3$ *S. aureus* at time 0, approaching from one side of the spine. At $t = 1$ h, one group (1 animal, 4 discs) received 0.1 mL intradiscal dose of vehicle as a control (50% v/v Omnipaque 300, 0.5% w/v hyaluronic acid, Water for Injections (WFI)) administered via the same side of the spine (co-localized with the bacteria). At $t = 1$ h, a second group (2 animals, 8 discs) received 0.1 mL intradiscal dose of linezolid administered via the same side of the spine as the bacteria (co-localized). At $t = 1$ h, a third group (2 animals, 8 discs) received 0.1 mL intradiscal dose of linezolid administered via the opposite side of the spine (discrete dosing). X-ray imaging of dose administration was used to identify discs in which a major dose leakage was observed, and these discs were excluded from analysis. At $t = 24$ h the animals were euthanized, the spine section T13 to L6 was removed and then the nucleus pulposus of each treated disc was dissected. The average weight of harvested nucleus pulposus tissue was ($n = 5$ per level), L1/L2 0.66 g, L2/L3 0.52 g, L3/L4 0.67 g, L4/L5 0.68 g, L5/L6 0.80 g. The isolated discs in separate labeled tubes were shipped on ice to Evotec (Manchester, UK) for microbiological assessment. Disc tissue was disrupted (Precellys Evolution, 2 cycles of 6000 rpm for 45 s) and bacterial burden was assessed as colony-forming units per gram of disc tissue (CFU/g) at 16 h of incubation at 37°C in aerobic conditions on Mannitol Salt Agar (MSA).

### 2.7 | Non-GLP systemic and intervertebral disc pharmacokinetics of PP353 development formulation

Intradiscal and systemic pharmacokinetics of PP353: Eight healthy Charollais cross, castrated male sheep, age <1-year, weight 43.75 ± 1.39 kg, were administered intradiscally 0.1 mL of a PP353 development preparation (linezolid 50 mg/mL) into the nucleus pulposus of 4 vertebral discs L1/L2, L2/L3, L3/L4, and L4/L5 providing a target linezolid dose of 5 mg/disc and a total of 20 mg intradiscal linezolid. Blood samples were collected, through a 5 French 20 cm cannula inserted into a jugular vein, in sodium heparin tubes, from live animals at $t = 0$, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 16 h, and 30 h and by jugular venepuncture at 48 h and 168 h. Plasma was separated by centrifugation (2000×$g$, 10 min, 4°C) and frozen. At time points $t = 0$, 24 h, 48 h or 168 h, the sheep were euthanized, and the intervertebral discs were excised, weighed, and frozen. The average weight of harvested nucleus pulposus tissue was ($n = 8$ per level), L1/L2 0.46 g, L2/L3 0.47 g, L3/L4 0.53 g, L4/L5 0.57 g. Nucleus pulposus tissue was macerated in phosphate-buffered saline. The nucleus pulposus and plasma samples were deproteinated by precipitation with acetonitrile. The non-compartmental pharmacokinetic parameters were estimated using Phoenix software version 1.4. The disc tissue collection took an average of 0.6 h.

### 2.8 | GLP pharmacokinetics and local tolerance of intradiscal and intramuscular administration of PP353

Twenty-seven Charollais cross, castrated male sheep were randomly assigned to one of nine groups of treatment with three animals assigned to each group. Three of the groups received intramuscular administrations in muscles adjacent to the vertebrae L2/L3 and L3/L4 and six received intradiscal administration of 0.1 mL of PP353 into 2 discs per animal at L2/L3 and L3/L4. In three of the groups receiving intradiscal administration, an additional disc was treated with a needle stick control sham procedure with no administration. The experimental design is described in Section S5.5 and Table S1. Additional groups for pharmacokinetics were dosed. There were no unscheduled deaths during this study.

On Days 1, 14, and 28 after treatment, animals from each group were euthanized, and treated discs and surrounding tissue were harvested. All tissues sampled were fixed in 10% neutral buffered formalin at room temperature for at least 5 days. Fixed tissue was transferred to a histopathology laboratory for the local tolerance assessment (Charles River, Edinburgh, UK). Bone samples were decalcified using 5% Nitric acid prior to processing for wax histology. A Board-certified Veterinary Pathologist provided qualitative assessment of the histology slides.

Linezolid pharmacokinetic parameters in the sheep were estimated using Phoenix (WinNonlin) Pharmacokinetic software (Certara, USA) version 8.3. The plasma linezolid after intramuscular administration was modeled as a zero-order input using a 2-compartment model. This analysis provided estimates of linezolid clearance and volume of distribution in sheep and provided the characteristic response for deconvolution analysis. Other PK analyses used a non-compartmental approach consistent with the extravascular (intramuscular and intradiscal) route of administration. The area under the linezolid concentration vs. time curve (AUC) was calculated using the linear trapezoidal with linear interpolation method. When practical, the terminal elimination phase of each concentration versus time curve was identified using at least the final three observed concentration values. The slope of the terminal elimination phase was determined using log-linear regression on the unweighted concentration data. Deconvolution of plasma linezolid after intradiscal administration enabled an estimate of linezolid remaining in the disc.[41]

## 3 | RESULTS

### 3.1 | Preliminary formulation studies

The properties of linezolid, with an aqueous solubility of less than 5 mg/mL, limited the dose of soluble drug that could be administered, and the target of 50 mg/mL linezolid was not achievable. Attempts to use cyclodextrin to increase the solubility of linezolid were partially successful, but high concentrations of cyclodextrin-solubilized linezolid formulations were not stable. An alternative approach, the preparation of a linezolid suspension of poloxamer and iohexol, was adopted. Linezolid is commercially available in two crystal forms: II and III. The two forms are characterized by their distinctive crystal melting points in differential scanning calorimetry or by their powder X-ray diffraction spectrum.[42,43] As supplied, both forms of linezolid comprised large crystals. Both crystal forms (1 g) were subjected to air jet milling to provide micronized powder. The particle size distribution of linezolid powder was analyzed by laser diffraction. Linezolid Form II as supplied had 90% of particles ($X_{90}$) at or below 44.7 µm. Post-milling, the $X_{90}$ was 3.95 µm. Linezolid Form III supplied had an $X_{90}$ of 11.4 µm and post-milling was 4.5 µm. It was not possible to create homogeneous stable suspensions of linezolid crystal form III as the micronized crystals clumped. In contrast, micronized linezolid crystal form II provided homogeneous suspensions that were stable for hours. When the linezolid crystal form II milling batches were scaled up from 0.5 kg to 2 kg, the milling was performed under nitrogen, and the milled linezolid form II $X_{90}$ was 4 to 5 µm. Although the suspensions were stable and homogeneous for use, for at least a 3-h period, the suspension was not suitable for long-term storage as the micronized powder did eventually clump. This led to the development of a two-vial approach. A vial of micronized linezolid powder and a vial containing a vehicle for suspension of the powder to be mixed prior to use. Sterile vials of 253 mg of micronized linezolid Form II powder were labeled PP353-A (Section S2).

Preliminary exploration of a range of vehicles for suspension of the linezolid led to the selection of a formulation containing poloxamer 407 and iohexol. A surprising interaction between the poloxamer 407 and iohexol led to a reduced solution-to-gel (sol/gel) transition temperature. Experiments testing a matrix of iohexol and poloxamer concentrations led to the selection of poloxamer 407 12.5% (w/w) with iohexol at about 150 mg iodine/mL (Section S6). The excipients used in Omnipaque™ 300 (calcium disodium EDTA and tromethamine) were used without further optimization. The suspension vehicle was

prepared on an 18.4 L (~22 kg) scale and sterilized by triple filtration in a sterile isolator, where it was packed in vials and then labeled PP353-B (Section S3).

The formulation of PP353, an investigational drug product for intradiscal administration, comprises two individually vialed drug components; 253 mg of micronized gamma irradiated linezolid Form II powder (PP353-A) and 7 mL of an aseptically filter sterilized suspension diluent (PP353B). The suspension diluent PP353-B contains iohexol, poloxamer 407, tromethamine, calcium disodium EDTA, and water for injection.

PP353 is prepared by adding 4.8 mL of PP353-B to a 253 mg PP353-A vial and shaking to create a homogeneous suspension for injection, giving a final concentration of 50 mg/mL of linezolid. The prepared PP353 was used within 3 h of mixing.

## 3.2 | In vitro characterization of release of linezolid

The release of linezolid was evaluated using Franz diffusion cells. Three preparations of linezolid Form II (un-milled with $X_{90}$ 44.7 µm and micronized batches with $X_{90}$ 8.45 µm and 3.58 µm) were assessed (Figure 1A). The rate of linezolid release was dependent on the particle size with smaller particles having a larger surface area that provides higher release rates.

To evaluate the effect of linezolid and poloxamer concentrations on release rates, four preparations containing linezolid at 50 mg/mL or 2 mg/mL and poloxamer at 12.5% (w/w) which gelled, or 6.25% (w/w) which was nongelling, at 37°C, were compared (Figure 1B).

The initial release profile for 50 mg/mL linezolid formulations demonstrates constant release into the receptor compartment independently of poloxamer concentration consistent with dissolution from a depot, maintaining the availability of soluble linezolid in the donor compartment. The initial release profile for the 2 mg/mL linezolid formulation demonstrates decreasing rate of release over time, consistent with the linezolid being fully soluble and the reduced donor compartment soluble linezolid concentration driving slower release into the recipient compartment, independent of poloxamer concentration. This demonstrates the depot effect and that there is no interaction between the linezolid and the poloxamer.

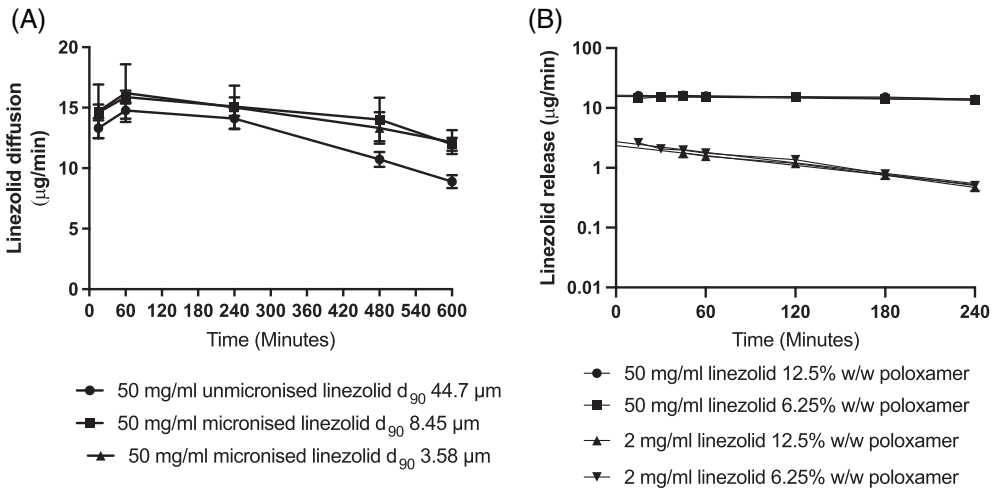


**FIGURE 1** (A) Effect of linezolid particle size on linezolid release (mean ± SD). (B) Effect of linezolid and poloxamer 407 concentration on linezolid release (mean ± SD).

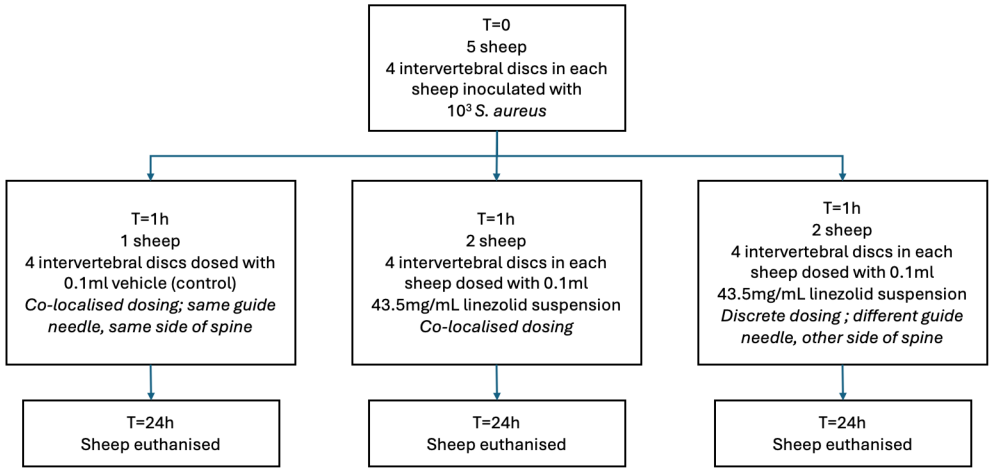


**FIGURE 2** Design of the sheep intradiscal infection efficacy study.

### 3.3 | Preclinical in vivo efficacy of intradiscal linezolid

In the sheep *S. aureus* disc infection model, discs were infected with $10^3$ *S. aureus* ATCC29213 at time zero and were treated at 1 h with vehicle control or with linezolid 4.35 mg/disc (Figure 2). PP353 was administered using a 25G 119 mm spinal needle confirming its suitability for percutaneous administration. Injectability through 22G 178 mm spinal needles was confirmed. Disc tissue was harvested at 24 h and bacterial burden assessed. The disc bacterial burden increased in the vehicle control group from $10^3$ inoculum to $10^8$ bacteria per gram of disc in 24 h (Figure 3). All seven of the evaluable discs in the co-localized linezolid treated group, in which bacteria and linezolid were administered via the same guide needle, were below the limit of detection, and potentially sterilized as bacteria were not recovered. One disc was not evaluable. Four of the six evaluable discs in the discrete dosing group in which bacteria and linezolid were administered via different guide needles from opposite sides of the spine, were also below the detection limit and potentially sterilized. The bacterial burden in the further two discs in the discrete dosing group was lower than controls. Co-localization of bacteria and linezolid is not required for efficacy. While efficacy of discretely dosed linezolid may be more variable than co-localized dosing, it was superior to discretely dosed vancomycin (see Figure S3, Section S8).

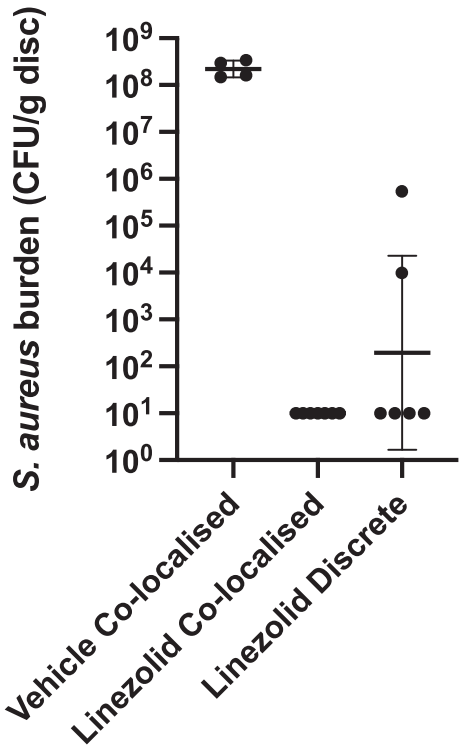


**FIGURE 3** In vivo efficacy of intradiscal linezolid. Bacterial burden values are geometric mean ± geometric SD. The potentially sterile discs were plotted as 10 CFU/g disc as an estimate of the limit of detection. The Mann-Whitney Rank Test *p*-values for colocalized linezolid and non-colocalized linezolid dosing compared to vehicle controls are 0.002 and 0.003 respectively.

### 3.4 | Non-GLP in vivo pharmacokinetics

The pharmacokinetics of PP353 was investigated after intradiscal injection in sheep by bioanalysis of plasma and vertebral disc samples after injection. Upon injection, PP353 was observed on X-ray imaging of the sheep disc (Figure 4). Linezolid in plasma and nucleus pulposus was estimated (Table 1; Figure 5A).

Four discs per sheep were each dosed with a target 5 mg of linezolid. The actual dose received was 4.55 ± 1.28 mg (mean ± SD). At the first time point, about 40 min after dosing, a mean nucleus pulposus linezolid content of 3.16 ± 0.79 mg/disc was estimated. This was considered a reasonable recovery of linezolid allowing an average 30 min diffusion from the disc and loss during in-life stage, dissection, and maceration of the disc. The intradiscal linezolid content fell exponentially over 48 h to 6.97 ± 6.91 μg/disc. The apparent linezolid $T_{1/2}$ in sheep discs is 5.54 h. Plasma linezolid concentration vs. time profiles were consistent with the extravascular dose route, in which post-dose elimination from the discs and an absorption phase in tissues up to 8 h after administration was evident followed by a monophasic decline in concentrations up to 48 h. Although 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 to disc concentrations. The apparent $T_{1/2}$ in plasma was 9.35 h.

### 3.5 | GLP pharmacokinetic analysis of PP353 in sheep

The intradiscal and intramuscular pharmacokinetics of a technical batch of PP353, manufactured at the same scale and process as subsequent clinical batches, was evaluated (Table 2). Two discs or the longissimus dorsi muscle layer adjacent to the intervertebral discs per animal were injected with linezolid at a target dose of 5 mg per injection site (0.1 mL PP353). The average actual dose of linezolid per injection site for intradiscal administration was 4.70 ± 0.45 mg and for intramuscular administration was 5.17 ± 0.19 mg. After single intramuscular administration of PP353 at two sites, plasma linezolid

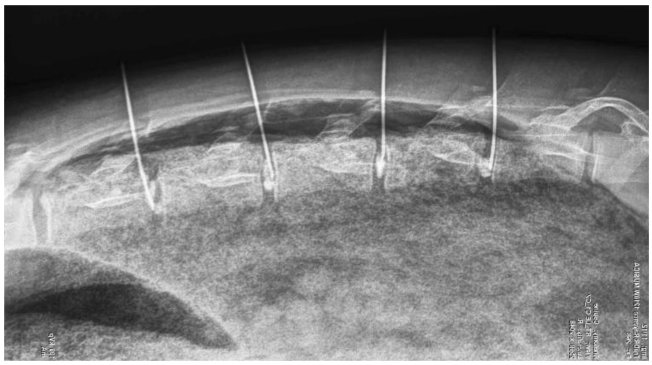

**FIGURE 4** Lateral view of the X-ray image of the sheep lumbar spine after intradiscal administration of PP353.

**TABLE 1** Pharmacokinetic parameters of linezolid in male sheep intervertebral disc and plasma after a single administration of a target of 5 mg/disc, 4 discs per sheep.

| | Target dose (mg/disc) | $T_{max}$ (h) | $C_0$ (µg/mL) | $C_{max}$ (SE) (µg/mL) | $C_{max}$/D (µg/mL)/(mg) | AUC(0-*t*) (SE) (µg.h/mL)/(mg) | AUC(0-inf) (µg.h/mL) | AUC(0-inf)/D (µg.h/mL)/(mg) | T1/2 (h) |
|---|---|---|---|---|---|---|---|---|---|
| Nucleus pulposus | 5 | 0.617 | 6980 | 6510 ± 839 | 1300 | 91 700 ± 10 500 | 91 800 | 18 400 | 5.54 |
| Plasma | 4 × 5 | 8 | - | 0.183 ± 0.013 | 0.009 | 4.91 ± 0.37 | 0.512 | 0.256 | 9.35 |

*Note*: Units in mL assuming 1 g = 1 mL. Disc harvest 0.51 ± 0.09 g (mean ± SD $n = 32$). For plasma estimates, the total target dose of 20 mg/animal (4 discs injected with 5 mg of linezolid) has been used for the calculation of the dose-normalized parameter. $T_{max}$, time of peak level $C_{max}$; $C_0$, extrapolated peak concentration at time zero; $C_{max}$, maximum observed concentration; $C_{max}$/D, $C_{max}$/Dose; AUC(0-*t*), Area under concentration-time curve to last measurable time; SE, standard error; AUC(0-inf), AUC zero to infinity; AUC(0-inf)/D, Area under concentration-time curve to last measurable time/Dose; T1/2, Half-life.

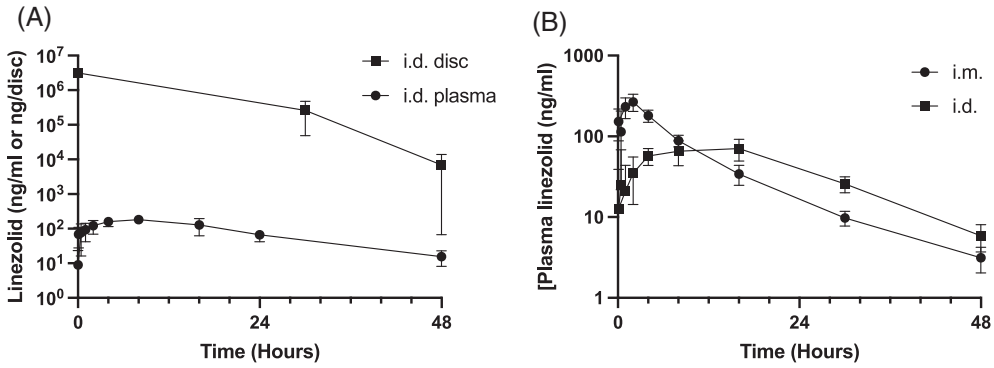


**FIGURE 5** (A) Pharmacokinetics of PP353 in plasma and nucleus pulposus (mean ± SD). Linezolid concentrations in plasma after intradiscal (i.d.) administration of linezolid (5 mg/disc, 4 discs per animal) to sheep. Plasma concentrations over the first 24 h are mean of 6 animals and at 48 h 4 animals. The intradiscal concentrations are mean of 8 discs, 4 discs in each of 2 animals. (B) Plasma pharmacokinetics of linezolid after Intradiscal and intramuscular administration of PP353 (mean ± SD). Linezolid concentrations in plasma after intradiscal and intramuscular administration of linezolid (5 mg, 2 discs or 2 i.m. sites per sheep; 10 mg total) to sheep. Plasma concentrations up to 16 h are mean of 9 animals and beyond 16 h the mean of 6 animals.

**TABLE 2** Summary of Sheep PK parameters.

| Route | $T_{max}$ (h) | $C_{max}$/D (ng/mL)/(mg/kg) | AUC(0-inf)/D[a] (ng.h/mL)/(mg/kg) | $T_{1/2}$[a] (h) |
|---|---|---|---|---|
| Intramuscular 5 mg linezolid 2 sites. Total dose 10 mg | 2 | 26.9 | 220 | 6.7 |
| Intradiscal 5 mg 2 discs. Total dose 10 mg | 8 | 8.1 | 221 | 6.7 |

*Note*: $T_{max}$, time of peak level; $C_{max}$/D, $C_{max}$/Dose; AUC(0-*t*), Area under concentration-time curve to last measurable time; AUC(0-inf)/D, Area under concentration-time curve to last measurable time/Dose; T1/2, Half-life.
[a]Estimates reported from animals sampled up to 48 h after dose only.

concentration versus time profiles were consistent with the intramuscular dose route, whereby a post-dose increase in concentrations was evident up to between 0.25- and 2-h after dose, followed by a generally monophasic decline in plasma concentrations (Figure 5B). Following intradiscal administration, plasma linezolid concentration versus time profiles shows a delay in linezolid distribution from the injection site into the systemic circulation, and a lower maximum linezolid concentration in plasma.

Systemic exposure to linezolid (based on dose-normalized AUC(0-inf)) was comparable between dose routes with a mean relative bioavailability (Frel) of 97%. However, $C_{max}$/D was 3.0-fold lower and Tmax was later after intradiscal administration compared to the intramuscular route, reflecting the slower rate of systemic input from the disc. No differences in mean $T_{1/2}$ between dose routes were noted.

The plasma linezolid concentration after intramuscular administration was modeled as a zero-order input using a 2-compartment model to estimate sheep linezolid volume of distribution at 35 L or 0.8 L/kg and clearance at 4.4 L/h and to deconvolute plasma concentrations after intradiscal administration thereby predicting the linezolid remaining in the disc in the non-GLP study (Figure 6).

There was correlation between the amount of linezolid measured in the disc and the amount estimated to remain in the disc by deconvolution of the plasma concentration over time. Although

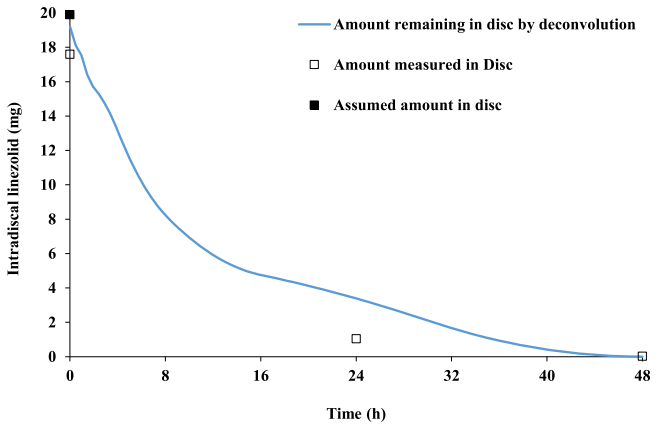


**FIGURE 6** Correlation between intradiscal linezolid concentration in the non-GLP study estimated by deconvolution of plasma concentration and that measured in disc tissues. Note that this is the estimate for 4 discs receiving the targeted 5 mg linezolid per disc.

deconvolution estimated higher disc concentrations at 24 h than measured, this may reflect the losses during harvesting of the nucleus pulposus. The profile indicated that approximately 50% of linezolid remained in the disc after 6 h.

### 3.6 | GLP local tolerance of PP353

Linezolid is an established antibiotic with a safe history of use. Local tolerance to a new site of administration, the intervertebral disc, was assessed.

Potential systemic effect of the procedure or PP353 was assessed by daily clinical observations for depression, appetite loss, dehydration, increased respiratory effort, and lameness. No abnormal clinical signs were observed throughout the 28-day observation period. Bodyweights were maintained within 10% of Day −1 weight throughout the study. A mild post-procedure pain response, observed as a slight reaction to palpation of the injection site, was seen in 7 out of 27 animals. The highest incidence was in groups receiving an intramuscular injection with 3/9 animals showing a mild response. The maximum duration of the pain response was 1 day, and all pain reactions had resolved within 2 days post-procedure.

Histological examination found that intradiscal needle insertion, either by administration of the drug product formulated with PP353, PP353-B diluent, or needle stick, was associated with a minor increase in cellularity of the nucleus pulposus on Days 1 and 28 and disorganization of the nucleus pulposus on Day 14. No increase in severity or incidence was observed in the formulated drug product or diluent treated discs compared to the needle stick-injured discs, suggesting that the changes were related to the injection process rather than the material delivered. Although these changes persisted until the end of the study, both the incidence and severity of the findings decreased over the course of the study and were largely of minimal severity by Day 28, suggesting that complete resolution may be seen with time. Minor hemorrhage in the tissues surrounding the ganglia of the spinal cord was observed in one animal at Day 7 and one animal at Day 14 subject to P353-B diluent and needle stick injection but was not seen in the Day 28 animals indicating that this was not a persistent finding.

Both intradiscal and intramuscular injections were associated with dark discoloration of subcutaneous tissue (bruising), subcutaneous hemorrhage, and dermal inflammation in the skin and skeletal muscle hemorrhage, and myofiber degeneration/necrosis. These findings were present on Day 1, Day 14, and Day 28, but without a clear relationship with the treatment group suggesting that they were procedural and related to the injection process rather than the test material. These findings persisted until the end of the study, but their incidence and severity decreased during the study and were of minimal severity on Day 28 suggesting that complete resolution may occur over time.

## 4 | DISCUSSION

Intervertebral disc bacterial infection is difficult to treat because the disc is usually not vascularized and systemic antibiotic therapy may not reach sufficient antibacterial exposure. This study provides a potential solution to this problem by describing a linezolid formulation suitable for percutaneous intradiscal injection, PP353.

Linezolid provides a broad coverage of Gram-positive bacteria that includes bacterial species frequently identified in disc tissue, has a safe history of use and a low incidence of resistance in clinical bacterial isolates.

The low aqueous solubility of linezolid presented an opportunity to create a suspension using micronized linezolid crystal form II powder. This suspension could potentially act as a depot, dissolving over time to provide extended exposure of linezolid in the disc. The suspension, prepared at the time of use, contained a vehicle with poloxamer 407 and iohexol. These components provided a liquid in which to make and administer a homogeneous suspension of the linezolid powder that on warming to body temperature the temperature-dependent gelling created a gel with high viscosity that would be less likely to leak from degenerate discs. The addition of iohexol facilitated image-guided administration.

The release of linezolid from PP353 was dependent on the size of the particles which altered the dissolution rate but not the poloxamer concentration as the release of gelling and nongelling formulations was similar.

Sheep were selected as the preclinical species because their larger spine anatomy was considered more relevant than other species.[44–46] A sheep bacterial disc infection model was developed, analogous to the mouse thigh model of bacterial infection.[47] Preliminary studies attempting to establish *C. acnes* infection in sheep discs indicated that there was no proliferation of *C. acnes* and the infection resolved naturally over several days. Intradiscal infection with *S. aureus* ATCC29213 demonstrated proliferation and maintenance of infection and was selected to evaluate the efficacy of linezolid formulations.

Intradiscal administration of linezolid to sheep discs infected with *S. aureus* was an effective treatment, potentially sterilizing most of the treated discs with a single administration. The maximum concentration of linezolid in the nucleus pulposus was about 10 000 times that observed in plasma following intradiscal injection of PP353. Comparison of the intradiscal and adjacent intramuscular pharmacokinetics of PP353 confirmed compartmentalization in the disc with a lower systemic $C_{max}$ and a later $T_{max}$. Once in systemic circulation, the elimination half-lives were the same. Intradiscal linezolid concentrations reduced considerably over 48 h but the restrictions on dose volume in sheep with intact, turgid discs meant that there is little opportunity to create a powder depot and the potency of linezolid means that an effective intradiscal exposure is maintained.

Deconvolution of linezolid plasma concentrations to estimate the amount of linezolid that had left the disc over time, and by subtraction, the amount of linezolid remaining in the disc was feasible and correlated with measurements of intradiscal concentrations. This supports the use of plasma pharmacokinetics to infer disc concentrations in clinical trials where intradiscal sampling is not feasible.

This study had limitations. The desire to minimize the number of sheep used in the in vivo studies impacted the ability to perform independent replicate assessments. To avoid creation of a severe pyogenic spondylodiscitis and to recapitulate a low bioburden chronic non-pyogenic bacterial disc infection a relatively low number of *S. aureus*, $10^3$, was administered. The efficacy of PP353 to treat disc infections with higher bacterial burden could be evaluated in the future and would be more indicative of efficacy in spondylodiscitis. Experimental animal model infections are usually allowed to establish for 2 h or more before administration of test antibiotics. The sheep had to be anesthetized for both bacterial infection and PP353 intradiscal administrations. Over 2 h of anesthesia or two periods of anesthesia within a day were considered burdensome for the animals and could potentially cause complications. A compromise of a single period of anesthesia and a 1-h period between bacterial infection and antibiotic treatment was adopted. This may lead to a less established bacterial infection that may be easier to treat. The sheep discs were healthy whereas patient discs tend to be degenerate and potentially fissured. Sheep discs could routinely only accommodate 0.1 mL of administered material because of administration back pressure. This limited dosing in sheep, but in patients greater volumes may be accommodated because of the larger anatomy and space created by degeneration and fissures.

In preparation for clinical evaluation of PP353, manufacturing was expanded and a technical batch of PP353 was prepared and used in a GLP local tolerance study in sheep. The findings indicated a local tissue reaction attributed to the intradiscal injection procedure itself rather than to linezolid or any of the formulation components and therefore local toleration of PP353 has been established. As intradiscal injection is a routine clinical procedure used in provocative discography and for administration of therapeutics, PP353 and its administration were considered to be suitable for clinical evaluation.

Ethics and Regulatory approval have been given to use PP353 in an ongoing Phase 1b clinical trial, Persica 002 (NCT04238676), "The Modic Trial" in the UK, multiple European countries and in New Zealand. The study will investigate safety, tolerability, pharmacokinetics, and efficacy in subjects with CLBP and Modic 1 or mixed Modic 1 and Modic 2. The first part of this study, characterizing the pharmacokinetics of a single intradiscal dose of PP353, has completed.[48]

## AUTHOR CONTRIBUTIONS

S.G. and L.C. initiated and co-led the study. L.C. provided the first draft of the manuscript. A.C.F. led early formulation development. G.H. and M.W. provided in vivo expertise, design, and execution of in-life studies. S.B., D.C., and A.W. provided ex vivo models and microbiological testing. P.J.C. led late-stage formulation development and CMC consultancy. J.H. led bioanalytical assay development and testing. C.B. provided pharmacokinetic analysis. A.B. led the design and provided pathology and histology expertise in support of the GLP local toxicology study. GH, SG, CB, and LC revised the manuscript following peer review. All authors edited and approved their sections and approved the manuscript in general.

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Persica Pharmaceuticals Ltd. funded the study and was responsible for the conceptualization, overall design, collation of data, decision to publish, and the first draft of the manuscript.

## CONFLICT OF INTEREST STATEMENT

SG and LGC declare a financial interest and salary from Persica Pharmaceuticals Ltd., a clinical-stage company developing intradiscally administered antibiotics to treat chronic low back pain (CLBP). Other authors work for contract research organizations contracted to provide expertise and services and their organizations received payment from Persica Pharmaceuticals Ltd. for the work detailed in this manuscript. Other authors declare no financial interests relevant to the manuscript.

## DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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