Bone and joint disorders: screening and early clinical drug development

Vrouwe, J.P.M.

CHAPTER 6

Safety, tolerability, pharmacokinetics and pharmacodynamics of single ascending doses of LRX712 in patients with knee osteoarthritis: a randomized placebo-controlled phase I trial

This chapter is a partial draft of the contemplated overarching paper, which will cover the pre-clinical and early clinical development of LRX712. Authors of a final paper are to be determined.

J.P.M. Vrouwe[1], F.E. Stuurman[1], J. Burggraaf[1,2], L.A. Coleman[3].

1 Centre for Human Drug Research, Leiden, NL
2 LACDR, Leiden, NL
3 Novartis Institutes for Biomedical Research, Cambridge, USA

ABSTRACT

BACKGROUND Osteoarthritis (OA) is a highly prevalent and debilitating disease, for which no disease modifying drugs are registered. LRX712 stimulates chondrocyte proliferation *in vitro* and in pre-clinical models. The objectives of this first-in-human (FIH) study were to evaluate safety, tolerability, and pharmacokinetics (PK) of single ascending doses of intra-articular (i.a.) injection of LRX712 into the target knee of patients with mild-to-moderate OA.

METHODS This double-blind, randomized, placebo-controlled, single ascending dose study included seven cohorts. At baseline, patients were randomized 4:2 to LRX712 (doses 0.5mg to 75mg) or placebo. During the 60-day follow-up period, patients were monitored for safety, tolerability, and PK.

RESULTS Forty-two patients were included; 28 were treated with a single dose of LRX712 and 14 received placebo. No serious adverse events occurred. The most frequently observed AEs were injection site joint pain or discomfort, which occurred more frequently in the higher dose groups. A pattern of transient elevations in high sensitivity C-reactive protein (hsCRP) post-administration was identified, primarily with dose levels of LRX712 > 15 mg. Pharmacokinetic analysis showed longer $T_{½}$ at higher dose levels, with large variation between subjects.

CONCLUSIONS The results of this first-in-human study indicated that LRX712 has a safety, tolerability and PK profile that supports further development including exploration of risk-benefit in the dose range most likely to be efficacious based on preclinical data. These clinical data suggest that intra-articular injection of LRX712 into the knee can be performed safely in patients with knee OA.

Introduction

Knee osteoarthritis (OA) is a highly prevalent and debilitating degenerative joint disease. The world-wide prevalence is estimated to be 13.5% in the population aged 40 years and over.[1] Its cause is heterogeneous and multifactorial: risk factors include a history of traumatic joint injury, obesity, aging, biomechanical factors, and hereditary factors.[2–4]

Clinically, knee OA manifests as joint pain, stiffness, swelling and loss of normal joint function.[5] The diagnosis is confirmed radiologically and characterized by joint space (cartilage) loss, osteophytes, subchondral bone marrow lesions and synovitis.[6,7]

Current therapies in knee OA are limited to symptomatic treatment which is achieved by lifestyle changes, patient education, local or systemic administration of analgesics or corticosteroid injections and – at a late stage – joint replacement.[5,8] As the safety and efficacy of currently applied analgesics (non-steroid anti-inflammatory drugs (NSAIDS) and opioids) and corticosteroids are limited, there is a clear need for disease modifying osteoarthritis drugs (DMOADS).[9,10] No approved chondro-protective or chondro-regenerative agents, capable of slowing down (or reversing) the degenerative process in OA, are available yet.[5,8,11,12] And several strategies for DMOAD development are currently researched.[13]

One approach toward disease modification is to stimulate cartilage formation. In healthy tissue, cartilage stem progenitor cells (CSPCs) differentiate into chondrocytes and form or repair (in cases where tissue is damaged) hyaline articular cartilage without induction of fibrosis, hypertrophy and ossification.[14] By mimicking pathophysiological pathways, the CSPCs may be stimulated to stop the degenerative progress in osteoarthritic cartilage and amplify the restoration of damaged cartilage.[14–16]

LRX712 is a compound that could be used for that purpose as it shows cellular differentiation of CSPCs and formation of hyaline cartilage both *in vitro* and in animal studies (unpublished data). LRX712 is a small molecule found by phenotypic screening. LRX712 is metabolized in the liver through N-oxidation into its major (inactive) metabolite, MAE344.

This first-in-human (FIH) study of LRX712 aimed to evaluate the safety, tolerability, and pharmacokinetics (PK) of single ascending doses of i.a. injection of LRX712 into the knee of patients with mild-to-moderate knee OA.

Materials and methods

STUDY DESIGN

This was a single center, FIH, double-blind, randomized, placebo-controlled, single ascending dose study, performed at the Centre for Human Drug Research, Leiden, The Netherlands. The study was approved by the medical ethics committee "Foundation Beoordeling Ethiek Biomedisch Onderzoek", Assen, The Netherlands, and was conducted in accordance with the Dutch law on research in humans. The trial was registered in clinical trial register clintrials.gov under NCT03355196.

Seven consecutive cohorts were studied, each with six patients with knee OA who received a single dose of LRX712, ranging from 0.5 mg to 75 mg, or placebo (4:2). After the i.a. injection, patients were observed in a clinical setting for four days; thereafter, follow-up visits took place on days 8, 11, 15, 22 and 29 with a final follow-up phone call on day 60.

For each dose level, sentinel dosing in the first two patients was applied (one active, one placebo). After blinded safety review of their five-day safety results, the remaining patients were dosed. Safety review- and dose escalation decisions were based on findings from physical examination, ECGs, vital signs, standard clinical laboratory evaluations (hematology, chemistry, urinalysis), adverse events (AEs) and available PK results.

PATIENTS

In a 35-day screening period, patients were screened for in- and exclusion criteria after obtaining written informed consent. Males and females, 30-65 years old, with radiologically confirmed mild-to-moderate knee OA (grades I-III according to Kellgren-Lawrence classification (KL) as determined on weight bearing X-ray within 6 months prior to enrollment), were enrolled.[6] The main exclusion criteria were a BMI <18 or >32 kg/m^2, significant abnormalities in blood pressure, 12-lead ECG, routine safety laboratory (hematology, chemistry, coagulation, virology) results, and the use of strong CYP3A4 inhibitors or inducers. Women had to be post-menopausal or surgically sterile and men had to use contraception. Patients were excluded in case of any i.a. treatment of the affected knee within 12 weeks prior to screening or if they used corticosteroids in any administration route other than topically. Paracetamol and NSAIDS could be used as rescue medication.

INVESTIGATIONAL PRODUCT

LRX712 and placebo (NaCl 0.9%; B. Braun Miniplasco) were administered by ultrasound guided i.a. injection. The doses of LRX712 were 0,5 mg, 2,5 mg, 5 mg, 15 mg, 25 mg, 40 mg, or 75 mg of LRX712 and all injections had a volume of 3 mL. As the appearance of LRX712 and placebo were different, the administrations were performed by an independent physician and the subject was blindfolded during the administration to maintain the blind.

ENDPOINTS

A full overview of the schedule of assessments is given in supplementary table 1.

Safety

The primary endpoint was safety, which included monitoring of AEs, physical examinations, vital signs, blood pressure, standard clinical laboratory evaluations (hematology, chemistry, urinalysis), and 12-lead ECG. Holter ECG-monitoring was applied from 24 hours before dosing until 96 hours post dose. In addition, a post-study analysis of hsCRP was performed using a latex-enhanced immunoturbidimetric assay by Siemens Advia Chemistry XPT (Siemens, Germany) with a reportable range of 0.16mg/L to 200.00mg/L for analysis of systemic outcomes.

Pharmacokinetics

For the PK evaluation of LRX712 and metabolite MAE344 in plasma, blood samples were obtained pre-dose, and at regular intervals post-dose (Supplementary table 1). After collection, the samples were centrifuged for 10 minutes at 4°C and 2000xg, and the separated plasma was stored at -70°C within 90 minutes after collection. When possible, synovial fluid sampling was performed pre-dose and after 4 days to explore the synovial fluid concentrations of LRX712 and its metabolite.

LRX712 and metabolite MAE344 concentrations were determined in plasma and synovial fluid using validated Liquid Chromatography with tandem mass spectrometry (LC-MS/MS) methods. The lower limits of quantification (LLQ) for LRX712 and MAE344 were 0.025 and 0.1 ng/mL for plasma, respectively, and 20 and 80 ng/mL for synovial fluid, respectively.

The plasma concentration-time profiles were analyzed using non-compartmental modelling using the software package Phoenix WinNonlin v.8.0

(Certara, Princeton, NJ, USA). The PK parameters for LRX712 included the maximal concentration (C_{max}), the time to reach maximal plasma concentration after drug administration (T_{max}), area under the plasma concentration curve until the last measurement (AUC_{last}), area under the plasma concentration curve to infinity (AUC_{inf}), terminal half-life ($T_{½}$), apparent volume of distribution (Vz/F) and clearance (CL/F). In addition, dose-normalized parameters were calculated for C_{max}, AUC_{last} and AUC_{inf}. For LRX712 metabolite MAE344, C_{max}, T_{max}, AUC_{last}, AUC_{inf}, and $T_{½}$ were calculated.

STATISTICAL ANALYSIS

The sample size was based on clinical considerations and the chance to observe AEs in this sample size. In case of an AE incidence of 33%, there would be an 80% chance of observing that AE within the 4 patients on active drug in one cohort, leading to the described group size. Safety and tolerability evaluation were based on descriptive statistics.

Results

PATIENTS

A total of 134 patients with knee OA were screened and 42 patients were included in the study; see CONSORT flow diagram in Figure 1.[17] The main reason for exclusion was uncontrolled high blood pressure at screening. All included patients completed the study. The demographic characteristics are summarized in Table 1. The randomized study population had a mean age of 57.1 years (range 30-65 years), 29 patients (69%) were female, and the mean BMI was 26.33 kg/m² (range 19.6-33.3 kg/m²). Most patients were Caucasian (92.9%).

ASSESSMENTS

Safety

An overview of the AEs observed during the study conduct, is shown in Table 2. No deaths, serious AEs or severe AEs occurred. A total of 34 patients (81.0%) experienced at least one AE, of which 27 patients received LRX712 (96% of LRX) and 7 patients received placebo (50% of placebo). The most reported AEs were injection site conditions (33 AEs in 20 patients, 47.6% of all randomized patients), headache (12 AEs in 12 patients, 28.6%) and back pain (8 AEs in 8 patients, 19.0%). Thirty-four patients (81%) had AEs of mild severity, of whom 6 patients (14.3%) also had an AE of moderate severity (5 patients had an injection site reaction and 1 patient had an episode of hyperventilation of 1 hr). The mild and moderate injection site reactions included discomfort, pain, stiffness, swelling, limited range of motion, hematoma, and dullness. All injection site conditions were self-limiting, and their duration varied from several hours to eight days.

A pattern of transient elevations in high sensitivity C-reactive protein (hsCRP) post-administration was identified, primarily with dose levels of LRX712 > 15 mg. Furthermore, occasionally out-of-range values were observed in vital signs, ECG, blood chemistry, hematology, and urinalysis, but no abnormalities of clinical significance were found during the study. In the 24-hour holter monitoring no clinically significant abnormalities, or dose related trends were observed either.

Pharmacokinetics

The plasma PK data of LRX712 and its metabolite MAE344 are shown in Figure 2 and summarized in Tables 3 and 4. LRX712 plasma concentrations above the LLOQ were measured in all dose levels. From the 2.5 mg dose level onwards, the plasma concentrations were above the LLOQ for at least one-week post-dose.

Both the C_{max} and AUC_{inf} increased up to 19.3 ng/mL and 1650 hr*ng/mL, respectively, after 75 mg LRX712. Plasma C_{max} seemed to plateau from 25 mg onwards, whereas a less than proportional increase of total exposure (AUC_{inf}) was still observed from 25 to 75 mg. A high inter-patient variability for plasma C_{max} and AUC_{inf} was observed, with coefficient variability values ranging between 54.1% and 107.4% for C_{max} and 32.7% to 66.4% for $AUC_{inf.}$ The time to maximum plasma concentration (T_{max}) was measured between 4 and 6.5 hours in the lower doses (up to 7.5 mg) and increased to a range between 14 and 24 hours for the higher dose levels between 15 and 75 mg.

The PK behavior (Figure 2) as observed in this FIH can be categorized in two types. In the first, observed in the low dose (≤7.5 mg) groups and in few patients in the higher dose levels, individual time–concentration profiles show a relatively fast decline of the plasma concentration of LRX712, with an apparent half-life shorter than 48 hours. In the second type, which was more dominant in the higher dose levels, the profile shows a plateau and slow decline of the plasma concentrations, leading to individual $T_{½}$ of more than 179 hours in the 15 and 75 mg dose levels. Since both categories occur in the dose levels ≥15 mg, a high variability is seen in the PK parameters, including mean $T_{½}$ in those groups

In the 40 mg group, the plateau-type profile of the plasma concentration was observed in two of the four patients. The determination of $T_{½}$ in these two patients was not possible due to the combination of the long $T_{½}$ and the sampling schedule. Therefore, the reported value for $T_{½}$ of this dose level (48.7 hours) is the mean of the two other patients and is an underestimation of the actual $T_{½}$ of this cohort.

The decline of the plasma concentrations in the four patients of the 25 mg dose group was faster than in the other patients who had a dose of ≥15 mg. This led to a $T_{½}$ in the 25 mg group of 32.8 ± 7.2 hours and matches the PK profiles observed in the ≤7.5 mg groups, rather than that of majority of patients in the higher dose groups.

LRX712 metabolite MAE344 was measured in plasma and exceeded the exposure of the parent drug. The maximum plasma concentration of MAE344 was reached after 7 to 72 hours. Depending on the dose level, C_{max} and AUC_{inf} were 5- to 12-fold and 12- to 18-fold higher than for LRX712, respectively (Tables 3 and 4).

Post-dose (72 hours) synovial fluid samples could be obtained in nine patients in the active groups. Concentrations ranged between 54.4 to 1900 ng/mL. The highest concentration was observed in the 75 mg dose group, but there was no clear relationship between local concentrations and dose level. The pharmacologically inactive metabolite MAE344 was not detected in synovial fluid.

Discussion

We report the results of a FIH clinical trial, which assessed the safety, tolerability and PK of single ascending doses of i.a. LRX712. The incidence of injection site reactions was higher in the active- than in the placebo group and AE incidence in the 40- and 75 mg dose groups was higher than that the lower dose groups. Moderate AEs only occurred in the 40- and 75 mg groups.

In the post-study analysis, transiently elevated hs-CRP was observed in 4/9 patients that experienced an injection site reaction in the 25, 40, and 75 mg dose groups. Of the patients who had an injection site reaction of moderate severity, two (33%) had elevated CRP. As such, ahs-CRP elevation was observed in some patients who had only mild local AEs and not in all patients who experienced local AEs of moderate severity. In pre-clinical studies (unpublished data) in beagle dogs, reversible inflammation of the knee joint synovium was observed in a high-dose subgroup of the treated animals, this may be in line with our clinical observations.

Although a difference in AE incidence between placebo- and active groups was apparent, the sample size of the dose groups was small, and results should therefore be interpreted with care. Also, post-procedural joint pain has a high incidence after the i.a. administration of other, registered compounds such as corticosteroids, with a post-injection pain incidence in the range of 33-50%, and a mean duration of 4 days.[18,19] In the clinic, i.a. injections are commonly combined with a local short-acting local anesthetic, which masks immediate post-injection pain. Such local anesthetics were not applied in this trial.

Although both the C_{max} (up to 25 mg) and AUC_{inf} of LRX712 increased with the dose, no definitive conclusions on dose proportionality can be drawn, due to the large interpatient variability of the PK profiles, in combination with the small sample size per dose level. Thus, the exact reason for the inter-patient variability is not fully understood and will be studied further. On the other hand, it should be considered that the systemic pharmacokinetics are of relative minor importance if it can be established that local concentrations of the drug remain high for a considerable and sufficient time to exert its effect. That said, it appears that high local concentrations of LRX712 lead to an increased AE incidence, which should be taken into account when investigating efficacious dose levels.

Although no effective DMOADs are registered, the search for new disease modifying agents aims at multiple pathways such as inhibition of IL-1-, TNF-α- WNT- or cathepsin K. So far, some positive effects have been observed regarding structural joint changes, but to date there are no studies that found a positive, clinically relevant effect on pain and/or functional outcomes.[22–24] This stresses the importance of measurement of these (patient reported) clinical outcomes in a very early stage of drug development.

One of the strengths of this study was the inclusion of a representable the patient population, that for which the compound is ultimately intended. Safety and tolerability of the i.a. injections- and the working mechanism of this compound may be subject to the disease state of the target joints. As LRX712 is most likely to have an effect in patients with mild-to-moderate knee OA, the outcomes of this study are thought representable for the target population. Another strength of this study was the extensive blood sampling for safety and plasma PK. The main limitation is the small sample size per dose level, which precludes firm conclusions about plasma PK behavior seen

in this trial. This study did not aim to investigate (long term) efficacy; hence, the follow-up duration is appropriate for the current study. Any study with the aim to investigate efficacy in OA would require a longer follow-up period, as disease progression is generally slow.

The results of this first-in-human study indicated that LRX712 has a safety, tolerability and PK profile that supports further development including exploration of risk-benefit in the dose range most likely to be efficacious based on preclinical data. These clinical data suggest that intra-articular injection of LRX712 into the knee can be performed safely in patients with knee OA.

REFERENCES

1. Cui A, Li H, Wang D et al. Global, regional prevalence, incidence and risk factors of knee osteoarthritis in population-based studies. *EClinicalMedicine*. 2020;29:100587. doi:10.1016/J.ECLINM.2020.100587
2. Wang Y, Teichtahl AJ, Cicuttini FM. Osteoarthritis year in review 2015: Imaging. *Osteoarthritis and Cartilage*. 2016;24(1):49-57. doi:10.1016/j.joca.2015.07.027
3. Blagojevic M, Jinks C, Jeffery A, et al.. Risk factors for onset of osteoarthritis of the knee in older adults: a systematic review and meta-analysis. *Osteoarthritis and Cartilage*. 2010;18(1):24-33. doi:10.1016/j.joca.2009.08.010
4. Lawrence RC, Felson DT, Helmick CG, et al. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. *Arthritis and Rheumatism*. 2008;58(1):26-35. doi:10.1002/art.23176
5. Kolasinski SL, Neogi T, Hochberg MC, et al. 2019 American College of Rheumatology/Arthritis Foundation Guideline for the Management of Osteoarthritis of the Hand, Hip, and Knee. *Arthritis Rheumatol*. 2020;72(2):220-233. doi:10.1002/art.41142
6. Kellgren JH, Lawrence JS. Osteo-arthrosis and disk degeneration in an urban population. *Annals of the rheumatic diseases*. 1958;17(4):388-397. doi:10.1136/ard.17.4.388
7. Sellam J, Berenbaum F. The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. *Nat Rev Rheumatol*. 2010;6(11):625-635. doi:10.1038/nrrheum.2010.159
8. McAlindon TE, Bannuru RR, Sullivan MC, et al. OARSI guidelines for the non-surgical management of knee osteoarthritis. *Osteoarthritis Cartilage*. 2014;22(3):363-388. doi:10.1016/j.joca.2014.01.003
9. Charlesworth J, Fitzpatrick J, Perera NKP, Orchard J. Osteoarthritis- a systematic review of long-term safety implications for osteoarthritis of the knee. *BMC Musculoskeletal Disorders*. 2019;20(1). doi:10.1186/s12891-019-2525-0
10. Schjerning AM, McGettigan P, Gislason G. Cardiovascular effects and safety of (non-aspirin) NSAIDs. *Nature Reviews Cardiology*. 2020;17(9):574-584. doi:10.1038/s41569-020-0366-z
11. Conaghan PG, Cook AD, Hamilton JA, et al.. Therapeutic options for targeting inflammatory osteoarthritis pain. *Nat Rev Rheumatol*. 2019;15(6):355-363. doi:10.1038/s41584-019-0221-y
12. Latourte A, Kloppenburg M, Richette P. Emerging pharmaceutical therapies for osteoarthritis. *Nature Reviews Rheumatology 2020 16:12*. 2020;16(12):673-688. doi:10.1038/s41584-020-00518-6
13. Vrouwe JPM, Burggraaf J, Kloppenburg M, et al. Challenges and opportunities of pharmacological interventions for osteoarthritis: A review of current clinical trials and developments. *Osteoarthritis and Cartilage Open*. 2021;3(4):100212. doi:10.1016/J.OCARTO.2021.100212
14. Jiang Y, Zhang W, Yin Z, et al. Human cartilage-derived progenitor cells from committed chondrocytes for efficient cartilage repair and regeneration. *Stem Cells Translational Medicine*. 2016;5(6):733-744.
15. Medvedeva E V., Grebenik EA, Gornostaeva SN, et al. Repair of damaged articular cartilage: Current approaches and future directions. *International Journal of Molecular Sciences*. 2018;19(8). doi:10.3390/ijms19082366
16. Decker RS, Um H Bin, et al. Cell origin, volume and arrangement are drivers of articular cartilage formation, morphogenesis and response to injury in mouse limbs. *Developmental Biology*. 2017;426(1):56-68. doi:10.1016/j.ydbio.2017.04.006
17. Schulz KF. Consort 2010 Statement: Updated Guidelines for Reporting Parallel Group Randomized Trials. *Annals of Internal Medicine*. 2010;152(11):726. doi:10.7326/0003-4819-152-11-201006010-00232
18. An TW, Boone SL, Boyer MI, et al. Effect of ice on pain after corticosteroid injection in the hand and wrist: A randomized controlled trial. *Journal of Hand Surgery: European Volume*. 2016;41(9):984-989. doi:10.1177/1753193416657678
19. Fawi HMT, Hossain M, Matthews TJW. The incidence of flare reaction and short-term outcome following steroid injection in the shoulder. *Shoulder and Elbow*. 2017;9(3):188-194. doi:10.1177/1758573217693808

Table 1 Patient baseline characteristics

	Placebo (n=14)	LRX712 0.5mg (n=4)	LRX712 2.5mg (n=4)	LRX712 7.5mg (n=4)	LRX712 15mg (n=4)	LRX712 25mg (n=4)	LRX712 40mg (n=4)	LRX712 75mg (n=4)	Full group (n=42)
Age at inclusion (years), mean (range)	56.1 (30-64)	58.5 (48-66)	59.3 (54-65)	50.0 (43-57)	57.5 (52-62)	60.5 (55-65)	58.8 (41-65)	58.8 (54-64)	57.1 (30-65)
Female n (%)	11 (78.6)	2 (50)	4 (100)	3 (75)	3 (75)	2 (50)	2 (50)	2 (50)	29 (69.0)
Race n (%)									
white	14 (100)	4	3	4	4	3	3 (75)	4 (100)	39 (92.9)
other	0 (0)		1 (25)	0 (0)	0 (0)	1 (25)	1 (25)	0 (0)	3 (7.1)
Height (cm), mean (range)	173.5 (1611.4-200.8)	173.88 (169.9-179.2)	167.8 (164.0-173.9)	176.0 (163.5-187.1)	171.2 (167.2-176.1)	173.7 (163.0-184.0)	168.7 (160.6-175.2)	177.6 (167.0-189.0)	172.9 (160.6-200.8)
Weight (kg), mean (range)	73.79 (55.2-98.9	80.95 (64.9-92.0)	78.25 (73.8-85.7)	80.78 (56.5-110.9)	78.82 (70.0-93.6)	88.1 (70.9-102.6)	75.43 (62.5-87.7)	89.93 (72.9-113.4)	79.1 (55.2-113.4)
BMI (kg/m^2), mean (range)	24.5 (19.6-30.3)	26.7 (22.5-29.9)	27.8 (27.4-28.3)	25.6 (20.0-31.5)	26.9 (23.4-30.2)	29.1 (26.7-33.3)	26.5 (24.2-30.5)	28.2 (25.2-31.7)	26.3 (19.6-33.3)
KL grade of the index knee n, %									
1	4 (29)	2 (50)	3 (75)	0 (0)	0 (0)	2 (50)	1 (25)	0 (0)	12 (29)
2	7 (50)	2 (50)	1 (25)	2 (50)	4 (100)	2 (50)	2 (50)	1 (25)	21 (50)
3	3 (21)	0 (0)	0 (0)	2 (50)	0 (0)		1 (25)	3 (75)	9 (21)

Table 2 Adverse events per treatment group

	Placebo (n=14)	LRX712 0.5mg (n=4)	LRX712 2.5mg (n=4)	LRX712 7.5mg (n=4)	LRX712 15mg (n=4)	LRX712 25mg (n=4)	LRX712 40mg (n=4)	LRX712 75mg (n=4)	Full group (n=42)
Numer of patients reporting an AE, n (%)	7 (50)	4 (100)	4 (100)	4 (100)	3 (75)	4 (100)	4 (100)	4 (100)	34 (81.0)
Patients reporting an AE of mild severity n (%)	7 (50)	4 (100)	4 (100)	4 (100)	3 (75)	4 (100)	4 (100)	4 (100)	34 (81.0)
Patients reporting an AE of moderate severity n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (75)*	3 (75)*	6 (14.3)
Patients reporting an AE of severe severity, SAEs or deaths n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Injection site conditions	3	4	4	3	2	2	8	7	33
Injection site (joint) pain	1	0	3	1	0	2	3	3	13
Injection site ROM impaired	0	0	0	0	1	0	3	2	6
Injection site swelling	1	0	1	1	0	0	2	1	6
Injection site reactions otherwise[1]	1	4	0	1	1	0	0	1	8
Musculoskeletal symptoms other than the target joint	3	0	1	0	1	5	0	0	10
Back pain	0	2	0	1	0	2	1	2	8
Gastro-intestinal AEs	0	0	3	1	2	1	0	0	7
Cardiovascular symptoms otherwise	1	0	0	2	0	2	2	0	7
Dermatological AEs	3	1	0	0	2	0	1	0	7
Respiratory tract AEs	1	3	0	1	0	0	1	1	7
Headache	1	0	1	1	1	0	1	1	6
Psychiatric AEs	2	0	0	0	1	1	0	0	4
Endocrine AEs	1	0	1	0	0	0	0	2	4
Urinary tract AEs	1	0	0	0	0	0	0	1	2

*AE adverse event, SAE serious adverse event, ROM range of motion / *AEs of moderate severity concerned injection site pain (in 3 patients of the 40mg group, 1 in the 75mg group), injection site movement impairment and hyperventilation (1 of each in the 75mg group) / 1 Injection site reactions otherwise include: joint discomfort (2 AEs), general injection site reaction, injection site dullness, injection site warmth and hematoma (all 1 AE).*

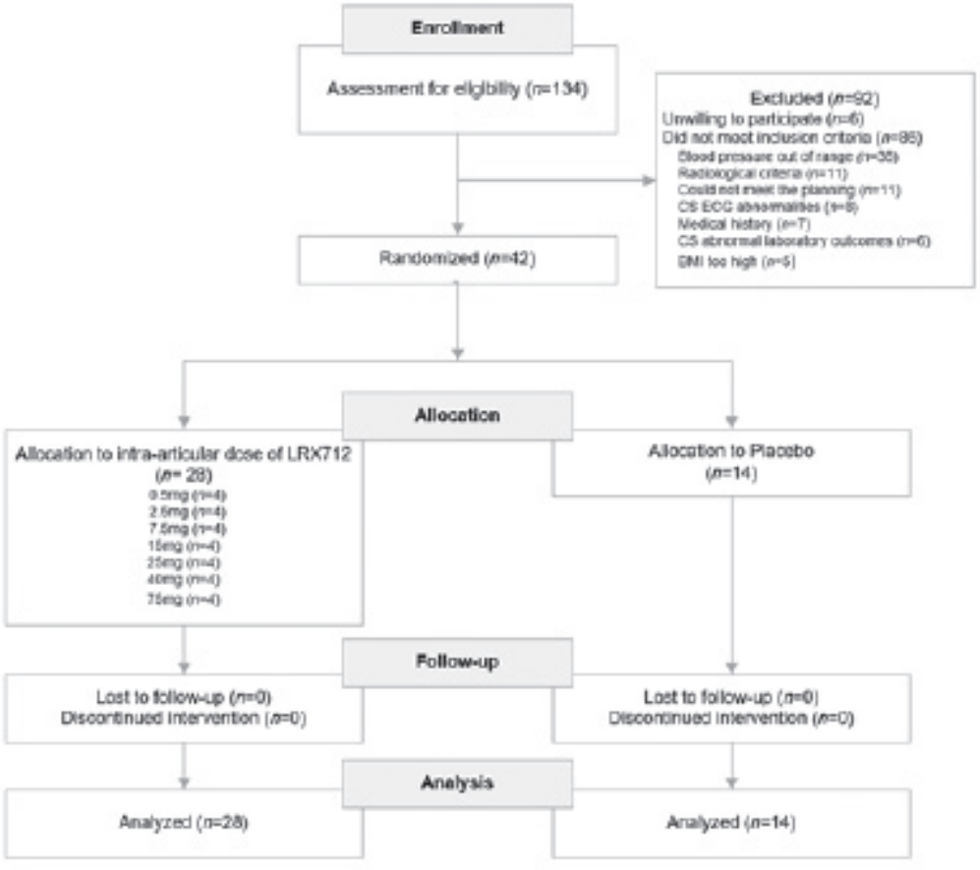
Table 3 Summary of plasma pharmacokinetics of LRX712 per dose level

	LRX712 0.5 mg (n=4)	LRX712 2.5 mg (n=4)	LRX712 7.5 mg (n=4)	LRX712 15 mg (n=4)	LRX712 25 mg (n=4)	LRX712 40 mg (n=4)	LRX712 75 mg (n=4)
C_{MAX} (ng/mL) Mean ± SD (CV%)	0.413 ± 0.329 (79.8)	2.27 ± 1.77 (78.1)	8.79 ± 6.24 (71.0)	3.85 ± 4.13 (107.4)	18.0 ± 4.25 (23.7)	17.6 ± 9.50 (54.1)	19.3 ± 11.7 (60.9)
T_{MAX} (hr) Median (range)	4.00 (4.00-4.00)	6.5 (4.00-24.0)	4.00 (4.00-4.00)	24.0 (4.0-24.0)	18.1 (8.0-24.3)	24.0 (8.0-24.0)	14.0 (4.0-24.0)
AUC_{LAST} (hr*ng/mL) Mean ± SD (CV%)	7.37 ± 3.87 (52.5)	67.5 ± 34.5 (51.1)	172 ± 116 (67.7)	335 ± 78.8 (23.5)	856 ± 282 (32.9)	1100 ± 393 (35.6)	1420 ± 840 (59.3)
AUC_{INF} (hr*ng/mL) Mean ± SD (CV%)	8.32 ± 3.82 (45.9)	69.4 ± 34.4 (49.5)	174 ± 116 (66.4)	405 ± 111 (27.4)	858 ± 281 (32.7)	1270 ± 585 (46.1)	1650 ± 1090 (66.1)
$T_{½}$ (hr) Mean ± SD (CV%)	23.2 ± 2.58 (11.1)	42.4 ± 31.4 (74.2)	27.4 ± 3.36 (12.3)	239 ± 160 (66.8)	32.8 ± 7.15 (21.8)	48.7 ± 31.1 (64.0)	179 ± 162 (90.4)
V_Z/F (L) Mean ± SD (CV%)	2430 ± 1280 (52.5)	2750 ± 2290 (83.4)	2210 ± 1100 (49.6)	12100 ± 7960 (65.6)	1530 ± 735 (48.1)	2110 ± 443 (21.0)	15000 ± 18100 (120.6)
CL/F (L/hr) ± SD (CV%)	70.3 ± 30.9 (43.9)	46.7 ± 30.2 (64.7)	58.5 ± 33.8 (57.8)	39.1 ± 9.98 (25.5)	31.5 ± 9.88 (31.3)	35.3 ± 16.3 (46.1)	61.1 ± 33.4 (54.7)

Table 4 Summary of plasma pharmacokinetics of MAE344 per dose level

	LRX712 0.5 mg (n=4)	LRX712 2.5 mg (n=4)	LRX712 7.5 mg (n=4)	LRX712 15 mg (n=4)	LRX712 25 mg (n=4)	LRX712 40 mg (n=4)	LRX712 75 mg (n=4)
C_{MAX} (ng/mL) Mean ± SD (CV%)	2.43 ± 1.65 (68.0)	10.2 ± 5.55 (54.2)	47.6 ± 20.8 (43.8)	45.4 ± 51.9 (114.4)	148 ± 25.6 (17.3)	199 ± 122 (61.1)	161 ± 85.9 (53.5)
T_{MAX} (hr) Median (range)	24.0 (8.0-24.0)	30.0 (8.0-36.0)	24.0 (12.0-24.0)	48 (24.0-54.0)	24.2 (24.0-30.0)	24 (24.0-48.0)	36 (24.0-72.0)
AUC_{LAST} (hr*ng/mL) Mean ± SD (CV%)	137 ± 58.0 (42.4)	767 ± 359 (46.8)	2950 ± 1390 (47.2)	5550 ± 826 (14.9)	10600 ± 1950 (18.3)	16900 ± 5750 (33.9)	21000 ± 11700 (55.7)
AUC_{INF} (hr*ng/mL) Mean ± SD (CV%)	147 ± 61.5 (41.9)	791 ± 428 (54.1)	2960 ± 1390 (47)	6790 ± 1440 (21.2)	10600 ± 1950 (18.4)	21300 ± 2650 (12.5)	23900 ± 14500 (60.8)
$T_{½}$ (hr) Mean ± SD (CV%)	37.4 ± 10.9 (29)	29.6 ± 3.08 (10.4)	33.3 ± 1.08 (3.2)	245 ± 155 (63.3)	37.6 ± 9.14 (24.3)	46.0 ± 21.1 (45.9)	159 ± 136 (85.2)

Figure 1 CONSORT 2010 flow chart of screened and included patients[17]



ECG electrocardiography, BMI Body mass index, CS clinically significant.

Figure 2 **PK graphs per cohort, with individual PK outcomes**