BMJ Open

Matrix-directed therapy losartan to identify the effect on the bone resorption marker carboxy-terminal crosslink of type I collagen telopeptide (CTX) in older adolescents and adults with osteogenesis imperfecta recruited from secondary care sites: the 'MOI-A' study; a randomised, phase 2/pilot, dose-escalating trial

Mahboobeh Haji Sadeghi ,[1] Judith Cohen ,[1] Bronwen Williams,[1] Amy Wilkinson,[1] Sarah Sumpter,[1] Laura Simms ,[1] Chao Huang ,[1] Keith Pugh,[2] Charlotte Heath ,[2] Luca Sangiorgi,[3] Francesca Gurioli ,[3] Maria Gnoli ,[3] Alice Moroni ,[3] Martina Piccinni Leopardi,[3] Clara Del Coco,[3] Nick Bishop [2,4]

To cite: Haji Sadeghi M, Cohen J, Williams B, *et al*. Matrix-directed therapy losartan to identify the effect on the bone resorption marker carboxy-terminal crosslink of type I collagen telopeptide (CTX) in older adolescents and adults with osteogenesis imperfecta recruited from secondary care sites: the 'MOI-A' study; a randomised, phase 2/pilot, dose-escalating trial. *BMJ Open* 2025;**15**:e107243. doi:10.1136/bmjopen-2025-107243

► Prepublication history and additional supplemental material for this paper are available online. To view these files, please visit the journal online (https://doi.org/10.1136/bmjopen-2025-107243).

Received 30 June 2025
Accepted 31 October 2025

For numbered affiliations see end of article.

Correspondence to
Dr Mahboobeh Haji Sadeghi; mahboobeh.hajisadeghi@hyms.ac.uk

ABSTRACT

Introduction Osteogenesis imperfecta (OI) is the most common inherited cause of bone fragility (approximately 1 in 16 000). People with OI suffer bone fragility causing fractures, pain and deformity; sarcopenia causing fatigue and poor endurance; aortic root dilatation and hearing loss. No drug currently has market authorisation to treat OI in Europe. Current standard-of-care is multidisciplinary, with pharmacological interventions—primarily bisphosphonates—directed at increasing bone mass; however, such interventions are of equivocal efficacy. The structural damage that can accumulate as a result of repeated fractures over time may not be reversible. The lack of a treatment with clearly defined efficacy in terms of reducing fracture frequency or the sarcopenia, that is increasingly recognised in this condition, leads to the consideration of alternatives based on what is known about the molecular pathophysiology of the condition. For reasons that are currently unclear, transforming growth factor beta (TGFβ) pathway signalling is increased in OI, and both studies in mouse models and more recently also in humans suggest that reducing TGFβ pathway signalling could be of benefit in OI. This demonstrator project tests the hypothesis that losartan, an antihypertensive agent known to reduce circulating TGFβ, will reduce bone turnover and bone loss and have a positive effect on muscle function and quality of life in adults and older adolescents with OI.

Methods and analysis This is a phase 2/pilot, open-label, dose-escalating study. This study aims to identify the effective dose for losartan in this population to inform the design of a pivotal phase III study. The study aims to recruit 30 adolescents and adults aged 16 years and above with OI across secondary care study sites in the UK and Italy. Participants will be recruited from the patient populations attending for treatment of OI at the participating hospital sites or referred by clinicians at the Participant Identification Centres (PIC sites). Participants will be randomised to one of three 'final doses'—25, 50 or 75 mg losartan once daily. All participants will start on 25 mg once daily. Those assigned to higher 'final doses' will increase in 25 mg once daily increments on day 8 and day 15 following safety assessments. The primary outcome measures are to establish the effective dose of losartan in OI patients, based on maximal reduction in

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Robust study design that will produce data to support the regulatory approval.
⇒ Statistical analysis was considered appropriate with excellent use of modelling for dose response in all stages of the study.
⇒ No conventional power calculations have been performed as this is a dose-determining study in a rare disease.
⇒ Small overall sample size (but similar to/greater than that of pharma-funded phase II studies in this condition).

the bone resorption marker carboxy-terminal crosslink of type I collagen telopeptide (CTX) over the 24-week period of the study.
Secondary outcome measures are to determine the changes in proxy efficacy outcomes for bone (turnover, mass, architecture and strength) using blood tests, high-resolution peripheral quantitative CT (HRpQCT), dual-energy X-ray absorptiometry (DXA) and muscle (strength) using the 'Timed Up and Go' test. In addition, the changes in quality of life, including pain and fatigue, will be evaluated by using a disease-specific tool (OI-QOL) and a validated generic tool (EQ-5D-5L-VAS).
Ethics and dissemination In the UK, the study protocol and amendments have been approved by the London Bridge Research Ethics Committee (REC reference: 23/LO/015) and by the Medicines and Healthcare products Regulatory Agency (MHRA). In Italy, the study protocol and amendments have been approved by the Italian and European ethics and regulatory authorities (Clinical Trials Information System European Union (CTIS EU) portal according to EU Regulation 536/2014). Final version of study protocol: Version 3.2, 05.03.2025. Final results will be disseminated in peer-reviewed journals through local OI, orthopaedic and other relevant clinical networks and at national and international meetings. Sheffield Children's National Health Service Foundation Trust (UK) and Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Istituto Ortopedico Rizzoli (Italy) are the joint study sponsors.
Trial registration number ISRCTN (ISRCTN13317811).

INTRODUCTION

Osteogenesis Imperfecta

Osteogenesis imperfecta (OI) is the most common inherited cause of bone fragility (approximately 1 in 16 000). People with OI suffer bone fragility causing fractures, pain and deformity; sarcopenia causing fatigue and poor endurance; aortic root dilatation and hearing loss. The range of severity is broad with severely affected individuals at risk of early death, for example, from respiratory failure in infancy, or progressively deforming bone disease that leaves them permanently wheelchair users with scoliosis, basilar invagination and intractable pain. Even the more mildly affected individuals have an increased risk of fracture and suffer with easy fatigability.

Current standard of care and potential benefits to health of our intervention

No drug currently has market authorisation to treat OI in Europe, with the only exception of neridronate in Italy. Current standard-of-care is multidisciplinary, with pharmacological interventions—primarily bisphosphonates[1]—directed at increasing bone mass; however, such interventions are of equivocal efficacy.[2] The structural damage that can accumulate as a result of repeated fractures over time may not be reversible. The lack of a treatment with clearly defined efficacy in terms of reducing fracture frequency or the sarcopenia, that is increasingly recognised in this condition, leads to the consideration of alternatives based on what is known about the molecular pathophysiology of the condition.

Existing pharmacological approaches to the treatment of OI, including those currently undergoing phase III studies, focus on increasing bone mass as a means to address the loss of bone mass, degradation of microarchitecture and alteration in bone material properties that render the bone brittle.

Recent studies in murine OI models showed increased transforming growth factor beta (TGFβ)-pathway signalling activity—cause unknown—and increased expression of TGFβ target genes.[3] Use of a murine pan-TGFβ neutralising antibody 1D11 in a severe OI mouse model (Crtap-/-) reduced bone resorption (carboxy-terminal crosslink of type I collagen telopeptide (CTX) biomarker 25%±5% lower) and significantly increased spine bone volume (235% increase in bone volume/total volume (BV/TV)).[3] The Col1a1Jrt/+ mouse showed no response, however, to 1D11.[4] Subsequently, use of a single dose of an anti-TGFβ antibody, fresolimumab, was associated with increased bone mass in a small study of adults with OI, although those most severely affected with type III and type VIII did not.[5]

Losartan is a drug used to treat hypertension, renal failure with proteinuria and cardiac failure and there are extensive real-world data on its safety and efficacy in these settings. Recent preclinical data show reduced circulating TGFβ, reduced bone turnover and increased bone mass in OI mice treated with losartan, and reduced TGFβ pathway signalling activity in OI osteoblasts treated with losartan.[6] Other preclinical data have shown that losartan-induced reduction in circulating TGFβ benefits muscle structure and function.[7]

We suggest that by decreasing circulating TGFβ levels through the use of losartan, we will both reduce bone turnover and bone loss, reducing fracture risk and need for orthopaedic interventions, and have a positive effect on muscle function and quality of life. This is a novel approach that has not been studied previously in a clinical setting.

METHODS AND ANALYSIS

Study aims and objectives

We have given careful consideration to the selection of both the primary and secondary objectives, in particular with regard to the relationship of the selected biomarkers with the desired outcome of fracture risk reduction as well as considering the short period of the study.

The primary objective is to establish the effective dose of losartan in 30 older adolescents and adults aged 16 years and above with OI, based on maximal reduction in the bone resorption marker CTX. The primary endpoint will be the percentage change in CTX from baseline to week 24.

Secondary objectives are to determine the changes in proxy efficacy outcomes for bone (turnover, mass, architecture and strength) using:

- Blood tests to measure the percentage change in CTX at week 8; as well as the percentage change in TGFβ and procollagen type 1 N-terminal propeptide (P1NP) at week 8 and week 24.
- High-resolution peripheral quantitative CT (HRpQCT) to measure the change in radial and tibial

total volumetric bone mineral density (vBMD) from baseline to 24 weeks. In this study, the HRpQCT scan will be performed as an optional assessment.
- Dual-energy X-ray absorptiometry (DXA) to assess the change in lumbar spine areal bone mineral density (LSaBMD) and hip from baseline to 24 weeks.
- The 'Timed Up and Go' test to measure muscle (strength) from baseline to 24 weeks.
- In addition, changes in quality of life (QOL), including pain and fatigue, will be evaluated by using a disease-specific tool (OI-QOL) and a validated generic tool (EuroQol 5-Dimension, 5-Level questionnaire and Visual Analogue Scale (EQ-5D-5L-VAS)). The change in OI-QOL and EQ-5D-5L-VAS from baseline to 24 weeks will be evaluated.

Study design

This is a phase 2/pilot, open-label, dose-escalating study. Final dose is randomly assigned. This study aims to identify the effective dose of losartan on CTX in this population to inform the design of a pivotal phase III study.

Study population

The study aims to recruit 30 adolescents and adults aged 16 years and above with OI. This is a pragmatic sample size given the rarity of OI. We would expect, based on our preclinical data and that of others, that individuals with OI of all ages might benefit from the use of losartan. Losartan does have a licence to be used in individuals from age 6 years and above, but we are being cautious in this preliminary study. Due to the lack of a licensed liquid formulation of losartan which thus precluded per kilogram bodyweight dosing, we decided to only include older adolescents. Losartan is licensed for the treatment of hypertension, chronic heart failure and renal disease in patients with type 2 diabetes mellitus with proteinuria. This licence applies in both the UK and Italy in accordance with the Medicines and Healthcare products Regulatory Agency (MHRA) and the Italian Medicines Agency (AIFA) as specified in the Summary of Product Characteristics (SmPC).

Participants will be randomised to one of three 'final doses'—25, 50 or 75 mg losartan once daily. All participants will start on 25 mg once daily. Those assigned to higher 'final doses' will increase in 25 mg once daily increments on day 8 and day 15 following safety assessments.

The proposed threshold for 'efficacy' in the determination of Optimal Biologic Dose (OBD) was set at a reduction in CTX of 30% to reflect our interpretation of preclinical data in relevant animal models and data from adult studies of osteoporosis, as data from adults with OI in respect of CTX are lacking. We do not know precisely what dose(s) of losartan might be effective in reducing circulating TGFβ and bone destruction as measured using CTX. We have preclinical data from a relevant mouse model demonstrating that a lower as opposed to higher dose of losartan was effective in reducing TGFβ and CTX. Over 20 years ago, a study in renal transplant patients demonstrated that losartan 50 mg/day reduced circulating TGFβ levels and improved allograft survival.[8] We have chosen doses around this 50 mg dose, in common use for the treatment of hypertension and renal failure with proteinuria, and hence known to be tolerable.

The preclinical data from two different model systems, one of which is milder, both indicated that a reduction in CTX was associated with a substantial increase in bone mass. In the more severe model system (crtap-/-), the reduction in CTX associated with treatment with an anti-TGFβ antibody for 8 weeks was 25% and was associated with almost complete restoration of vertebral bone mass (235% increase) and architecture.[3] In our studies of the milder model (OIM) treated with losartan 0.6 g/L in drinking water (equivalent to 48 mg/kg for the mice), the reduction in CTX was 40% and associated with a sixfold increase in bone mass compared with placebo. This dose was chosen as it was the same as that reported for a number of studies focusing on the effects of losartan on muscle outcomes.[7] Depending on the modelling approach used to 'translate' mouse to human doses, such a dose could equate to a dose within the range used to treat hypertension but might also substantially exceed it. Calculation based on surface area scaling as per a recent article from Nair and Jacobs[9] suggests this dose equates to 4.4 mg/kg in humans, more than three times the maximum dose used in clinical practice in humans. Other studies of the antihypertensive effect of losartan in mice have used a lower dose of 10 mg/kg, which would scale to 0.92 mg/kg. There is thus significant uncertainty regarding both inter-species scaling and ascertaining a dose to give sustained long-term decrease in CTX which is associated with improved bone density while minimising side effects.[10]

Study recruitment

Participants diagnosed with OI will be recruited across the UK and Italian study sites including Sheffield Children's Hospital, Sheffield Teaching Hospital, Royal National Orthopaedic Hospital, Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Istituto Ortopedico Rizzoli Bologna (Bologna), Policlinico di Milano Ospedale Maggiore | Fondazione IRCCS Ca' Granda (Milano).

Participants will be recruited from the patient populations attending for treatment of OI at the participating sites or referred by clinicians at the Participant Identification Centres (PIC sites). If potentially eligible, the site principal investigator (PI) and team will send them a Participant Information Sheet (PIS) containing details of the study.

For the UK sites, the site study team will provide the PIS and consent forms in English. Where a non-English speaker is identified as eligible for the study and there is sufficient time, translated written information (PIS and Informed Consent Forms) will be provided where possible and practicable (we have access to translational facilities through the Repurposing of Medicines 4 All (REMEDi4ALL) consortium). In addition, if translated

documents are not available, a local National Health Service (NHS) Trust interpreter may be requested by the potential participant to attend study discussions and informed consent visits with the participant.

For Italian sites, the study teams will have a translated version of the master PIS approved by the Italian regulatory authorities. However, where a non-Italian speaker is identified as eligible for the study and there is sufficient time, translated written information (PIS and Informed Consent Forms) will be provided where possible and practicable via the REMEDi4ALL consortium.

The site PI and research team will check eligibility of participants by searching any relevant database of patients attending hospital with OI, or from personal knowledge of patients. Full eligibility will be confirmed once informed consent has been taken by an appropriately delegated study doctor so that additional screening interventions can be undertaken.

Patients will be screened using the history of their OI, supported by any available clinical correspondence according to the usual standard of care. Eligibility checks include blood tests (Full Blood Count, Urea & Electrolytes and Liver Function Tests) and urine pregnancy test for women of childbearing potential (WOCBP).

All participants will be enrolled into the study REDCap Cloud (RCC) database immediately after consent to generate a pseudonymised Subject ID Number. Copies of all completed consent forms will be uploaded into the study database to enable central remote monitoring of the consent process.

Eligibility criteria

Inclusion criteria

- Age 16 years and above.
- Diagnosed with osteogenesis imperfecta (any type).
- Prior treatment with up to and including 6 weeks of oral bisphosphonate therapy is allowed provided there has been a 12-month washout period since the last dose of treatment.
- Prior treatment with a single dose of an intravenous bisphosphonate is allowed provided there has been an 18-month washout period since the treatment was given.
- Prior treatment with more than 6 weeks of oral or more than one single intravenous bisphosphonate therapy is allowed provided there has been a 10-year washout period since the last dose of treatment.
- Prior treatment with more than a single dose of denosumab is allowed provided there has been a 1-year washout period since the last dose of treatment.
- A woman of childbearing potential (WOCBP) who agrees to use an effective method of contraception from point of signing the informed consent throughout the study.
- Agreed not to participate in another interventional research project during their involvement in this study.
- Not taking prohibited concomitant medications, listed in exclusion criteria.
- Does not have any other contraindication that makes the patient unsuitable to take part in the study in the opinion of the investigator.

The inclusion criteria are based on the following considerations:

1. Age 16 years and above for the following reasons:
   a. The participant needs to be able to report on feelings of dizziness or unsteadiness that might occur following losartan administration.
   b. The patient group input was that it was appropriate to include participants within this age bracket.
   c. We anticipate future studies in children and will use safety data from this study to help inform dose considerations in children.
2. Diagnosed with OI based on established clinical criteria, supported by clinical history and physical examination findings. This is the disease group we wish to target, where preclinical data suggest that the intervention may have a beneficial effect in reducing bone turnover in OI and improving muscle function in multiple sarcopenic or myopathic models. OI is a clinical diagnosis based on the presence of fragility fractures as well as other clinical findings including, but not limited to, hypermobility, musculoskeletal pain, sarcopenia, blue sclerae and reduced stature.
3. Losartan is an angiotensin II receptor type 1 (AT1) receptor blocker and can have teratogenic effects if administered in early pregnancy, hence the need for female participants of childbearing age/potential to take contraceptive measures for the period of the study.
4. Involvement in another interventional project is not generally permitted as the effect of one intervention can interfere with the assessment of the other.
5. Avoidance of other drugs that can contribute to known side effects or issues with losartan, for example, including but not restricted to other antihypertensive agents, potassium-retaining or potassium-elevating drugs.
6. Prior treatment with bone-targeted drugs may impact on current bone metabolism. Available evidence from adult patients with osteogenesis imperfecta is lacking in respect of bone turnover following administration of bisphosphonates and denosumab; in children, bone turnover 'bounces back' after bisphosphonate administration within 12 months (depending on the drug and route of administration). Bone turnover is slower in adults than in children, but it is reasonable to expect that bone turnover should not still be suppressed 10 years after treatment had ceased. Rapid offset of denosumab is well recognised in both adults and children with rebound/overshoot reported both during trials and in clinical practice; denosumab does not persist in bone for more than 6 months at standard dosing.

Exclusion criteria

- Current use of losartan.

- Prior use of losartan within preceding 6 months to enrolment.
- Presence of other chronic illnesses, including renal failure likely to affect bone metabolism or structure.
- Known severe hypotension resulting in dizziness, fainting or headaches.
- Hyperkalaemia.
- Current medication that increases potassium retention, or may increase potassium levels, such as potassium-retaining diuretics.
- Current medication with lithium.
- Current medication with other substances which may induce hypotension.
- Currently taking oral bisphosphonates or intravenous bisphosphonates.
- Prior treatment with more than 6 weeks oral bisphosphonates treatment within 10 years of the consent.
- Prior treatment with more than a single dose of intravenous bisphosphonate within 10 years of the consent.
- Prior treatment with more than one dose of denosumab within 1 year of the consent.
- Recent (last 12 months) or current treatment likely to affect bone—this does not include inhaled or intermittent oral therapy with steroids for asthma (no more than 3 months of oral steroids in previous 12 months).
- Severe hepatic impairment (aspartate aminotransferase (AST)≥144 U/L, alanine aminotransferase (ALT)≥165 U/L, gamma-glutamyl transferase (GGT)≥183 U/L).
- Renal impairment (glomerular filtration rate (GFR)<60 mL/min/m2; GFR in children will be assessed using the Bedside Schwartz equation) if treated with aliskiren-containing products.
- Diabetes mellitus if treated with aliskiren-containing products.
- Cardiac failure if treated with diuretics (excluding grade 1 according to the New York Heart Association (NYHA) Functional Classification).
- Pregnancy or lactation.
- Known hypersensitivity to losartan or any of the excipients.
- Recent fracture in the prior 6 months to enrolment.

The exclusion criteria are based on the following considerations:

1. Current use of losartan.
2. Prior use of losartan within preceding 6 months to enrolment.
3. Presence of other chronic illnesses likely to affect bone metabolism or structure. Although unusual, other chronic diseases can be present in OI; given the limited objectives for the study in terms of defining OBD for losartan and identifying the size of proxy responses, we wish to reduce the likelihood of confounding any of the outcomes.
4. Hypotension is a potential complication of treatment with losartan; symptomatic hypotension is likely to be worsened with treatment.
5. Hyperkalaemia occurs in some individuals treated with losartan; a baseline potassium above the upper limit of the age and gender-specific reference range is a dose-reduction criterion; hence, a patient should not start treatment if they already meet this criterion.
6. Not treated with bisphosphonates or denosumab, or with minimal prior treatment, or a sufficient washout period of 10 years for bisphosphonates and 1 year for denosumab. The primary clinical outcome for the study is the determination of the 'effective dose' of losartan, assessed by the reduction in the bone resorption marker CTX. Bisphosphonates and denosumab act by reducing bone resorption, and thus, change in CTX may be affected by a prolonged period of prior treatment occurring in the recent past. As things stand, we have identified over 150 eligible participants who are completely naïve to treatment, so we may not have to recruit any that have had prior treatment.
7. Medications affecting bone could also affect the CTX response to treatment with losartan, either increasing CTX or reducing it within the time period of the assessments.
8. Severe hepatic impairment as per SmPC.
9. Significant renal impairment requires careful monitoring as per SmPC.
10. Cardiac failure requiring use of diuretics requires careful monitoring as per SmPC.
11. Pregnancy is an absolute contraindication to the use of losartan; lactation alters bone turnover.
12. Exposure of participants to a substance known to result in a hypersensitivity reaction is clearly inappropriate.
13. Recent fractures can elevate CTX levels for up to 6 months.

OI severity is not a specific inclusion or exclusion criterion, but naivety/very low exposure to prior bisphosphonate exposure will mean that most participants will fall at the milder end of the spectrum of severity.

Randomisation

All 30 participants will be randomised sequentially and the responsible statistician will prepare a randomisation schedule using the block randomisation with variable block sizes in a 1:1:1 ratio so that 10 participants will be randomised to each final dose arm that is, 10 randomised to final dose of 25 mg, 10 participants to 50 mg and 10 participants to 75 mg. Given that this is a dose escalation study, both the participant and the investigators will be aware of the dose that the participant receives.

Randomisation will be completed via the RCC (http://www.redcapcloud.com/) online system provided by the Hull Health Trials Unit (HHTU).

Study Interventions

A summary of the study intervention schedule outlined in online supplemental table 1 (see separate document upload).

Screening and baseline visits

The screening visit will take place at the hospital between day −28 and day 0 in order to confirm eligibility and will include the following interventions: informed consent, medical history, fracture history, physical/clinical exam, body mass index (BMI) (weight/height),[2] vital signs (temperature, pulse, respiration, blood pressure and oxygen saturations), urine pregnancy test for women of childbearing potential (WOCBP), and blood test (full blood count (FBC), clinical chemistry (U&Es), liver function tests (LFTs)). GFR in children will be assessed using the Bedside Schwartz equation. ECG will be performed at screening and whenever deemed necessary by the investigator.

Once eligibility is confirmed, the baseline visit assessments will be completed on day 1. All participants will be randomised at day 1 to their final dose allocation of losartan 25 mg, 50 mg or 75 mg once daily. However, all participants will start on the lowest dose (25 mg) and increase (if randomised to a higher dose) via a dose escalation pathway as described below. At this visit, participants will take their first dose of study Investigational Medicinal Product (IMP) while in the site. In order to monitor vital signs and check for adverse events and reactions, this visit will take up to 4–6 hours.

Other baseline investigations include: HRpQCT scan radius and tibia (optional), DXA of lumbar spine and hip, 'Timed up and go' test, pubertal stage assessment (for patients under 21 years old), QoL questionnaires (OI-QOL and EQ-5D-5L-VAS), blood tests (FBC, U&Es, LFTs, CTX, losartan, TGFβ, P1NP, bone profile (25-hydroxyvitamin D (25 OHD) and parathyroid hormone (PTH))). Fasting blood tests will be collected to measure losartan serum levels in order to assess drug concentration in participants across the different randomised arms. These will help us evaluate treatment efficacy and potential correlations between serum concentration and clinical outcomes.

The HRpQCT scans will be conducted at a central scanning site in each country. HRpQCT scan of the radius and tibia will be undertaken. Participants sit in a special chair and place either their wrist or ankle into a cylindrical aperture, keeping it very still for the duration of the scan which is around 3–4 min. A supportive splint is provided to help with this.

DXA lumbar spine and hip scans are a standard way of assessing bone density in both children and adults; participants lie on a couch and the scan arm passes above them to provide a quantitative assessment of bone size and mass. Scans take about 1 min each.

Dose escalation / modification visits

The study visits at weeks 1, 2, 3, 4 and 5 may be done at a clinic visit or a home visit, if a home visit service is available at the recruiting site. A visit window of ±1 day is permitted to schedule these visits. Figure 1 outlined the dose escalation/modification process.

The research team will complete the visit assessments including dose escalation in line with the dose escalation algorithm and assigned randomisation group, or modified in line with safety checks conducted at the weeks 1, 2, 3, 4 and 5 visits. All participants will start at a dose of 25 mg and doses may escalate (if randomised to a higher dose) or reduce depending on dose modification safety assessments. A safety assessment will consist of both a serum potassium check and a hypotension assessment. Both have the potential to modify the dosing schedule and both will be conducted before a decision to maintain, escalate or reduce dose is taken.

On day 7±1, a safety assessment check will be conducted on all participants. If both safety assessments are within acceptable limits and the participant was randomised to either the 50 mg or 75 mg groups, the losartan dose will be increased to 50 mg on day 8. On day 14±1, a safety assessment check will be conducted on participants taking 50 mg. If both safety checks are within acceptable limits, the participant will either remain on 50 mg (if randomised to 50 mg), or increase to 75 mg (if randomised to 75 mg group) on day 15. On day 21±1, a safety assessment check will be conducted on participants taking 75 mg. If both safety assessment checks are within acceptable limits, the participant will remain on 75 mg. On day 28±1, we will re-evaluate tolerance specifically and all participants will have a safety assessment. If the safety assessment is acceptable, the participant will continue on their allocated dose to the end of the study (week 24).

If at any of the dose modification visits the potassium level is greater than upper limit of normal (ULN) and/or the participant is experiencing persistent signs of hypotension, the dose will be reduced by 25 mg to a lower dose or if already on 25 mg the participant will be withdrawn from the study. For those who reduced their dose by 25 mg, a safety assessment will be repeated after 1 week. If the safety assessment checks are within acceptable limits, they will remain on the current dose for the remainder of the study (week 24), regardless of their original randomisation group on day 1. Participants with unacceptable safety assessments that withdrawn from the study will be referred for further management of their raised serum potassium or persistent hypotension.

Study follow-up visits

Day 42±1 visit is a phone call for all participants to record any adverse event or changes in concomitant medications.

Day 56±1 visit is to re-evaluate tolerance specifically and all participants will have a safety assessment. 'Timed up and go' test, pubertal stage assessment (for patients under 21 years old), quality of life questionnaires (OI-QOL and EQ-5D-5L-VAS) and blood tests (FBC, U&Es, LFTs, fasting CTX, losartan, TGFβ, P1NP, bone profile (25 OHD and PTH)) will be repeated at this visit.

Day 84±1, day 112±1 and day 140±1; these visits are simply to check physical well-being.

Day 168±1 is the final visit at which point study treatment will be discontinued. HRpQCT scan radius and tibia (optional), DXA LS and Hip, 'Timed up and go' test, pubertal stage assessment (on patients under 21 years

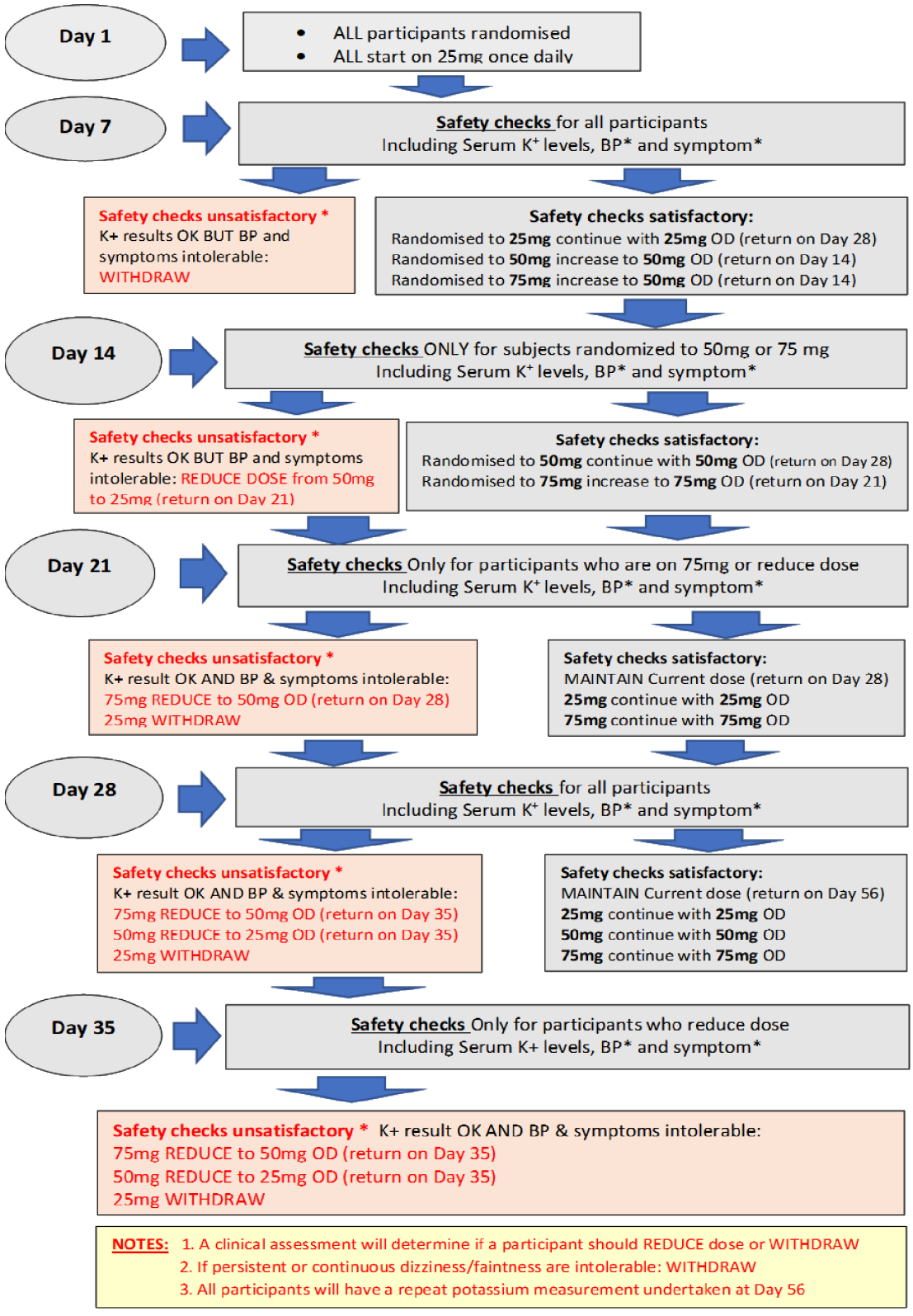

Figure 1 Dose escalation and modification; Window of ±1 day for each visit. *Blood pressure and symptom assessments will be conducted to check for hypotension. *A safety assessment will be conducted for all participants. BP, blood pressure; OD, once a day.

old), quality of life questionnaires (OI-QOL and EQ-5D-5L-VAS) and blood tests (FBC, U&Es, LFTs, fasting CTX, losartan, TGFβ, P1NP, bone profile (25 OHD and PTH)) will be repeated at this visit.

There are no follow-up visits after the final visit. All patients will remain under regular review with their treating physician and will be able to contact them should they notice anything untoward. Losartan and its

metabolite will disappear from the participant's system within 2–3 days after the last dose of treatment. Under normal clinical use circumstances, there is no requirement to either gradually reduce the dose or provide specific follow-up.

Early withdrawal visit

Participants who decide to leave the study or who are withdrawn will be asked to attend an unscheduled early withdrawal visit (scheduled for 1 to 3 days after last dose (ALD)) to assess patient safety and collect surplus drug supplies. If a participant decides to withdraw at or after week 12, it will be optional to do the HRpQCT radius and tibia scan, DXA LS and hip and 'Timed up and go' test. For earlier withdrawal, it is not necessary to repeat these tests.

Unscheduled visit

Based on existing evidence for the general use of losartan, if serum potassium has not risen within a week of either starting or escalating the dose, it is unlikely to do so. However, if at any stage there are problems with symptoms likely to be attributable to losartan, the patient is reviewed in their local centre and, if necessary, the dose is reduced to a lower dose and the patient reassessed after 1 week, and if they are on 25 mg, they are withdrawn at that stage.

Sample size

Data from a total of 30 participants will be used to derive a final estimate of the OBD. This will be a pragmatic sample size given the rarity of OI. No conventional power calculations have been performed. Up to six participants will be replaced following early withdrawal events, allowing for a 20% attrition rate.

Statistical analysis

We will recruit 30 participants into this study, who will be randomised into one of three doses (25, 50 and 75 mg once daily). The study's primary outcome will be reduction in CTX over the 24-week period of the study. The multiple comparison procedure-modelling (MCP-Mod) method will be used for finding the optimal dose.[11] The MCP-Mod approach is a hybrid approach that combines hypothesis testing and modelling, offering a modelling-based quantitative approach to dose selection. It overcomes the shortcomings of traditional dose selection methods and provides the flexibility of modelling for dose estimation, while preserving the robustness to model misspecification associated with MCP. This approach has been qualified by regulatory agencies (eg, European Medicines Agency (EMA) in 201416 and Food and Drug Administration (FDA) in 201617) as an efficient statistical method for phase II dose-finding studies when there is uncertainty about dose-response relationship.

The MCP-Mod results will be reported to the Data Monitoring and Ethics Committee (DMEC) and seek their advice on whether the selected candidate model is clinically meaningful. If none of the candidate models are appropriate, we will instead report the optimal dose based on the maximum average change in CTX from baseline to week 24.

A set of prespecified candidate models will be used to assess the presence of a dose response signal (MCP-step). Then, the optimal dose will be selected based on the parametric modelling (Mod step). We will repeat this analysis at 24-week follow-up, in order to assess the maximum reduction in CTX at long-term follow-up. The analysis will be undertaken by DoseFinding package in R version 4.3, or the latest available version.

In terms of study safety/tolerability outcomes, we will summarise each safety event during the 24-week follow-up period by dose group. Additionally, we will report the number/percentage of patients who cannot achieve the allocated dose (particular for the 50 and 75 mg dose). For safety monitoring purposes, we will periodically report to DMEC at pre-specified timepoints about the patient dose calculation algorithm and reasons for 'no escalation' decisions (potassium and hypotension). If treatment with 25 mg losartan is not tolerated by the majority of the participants, then the DMEC will review data and provide recommendations on protocol modifications or study continuation to the Trial Steering Committee (TSC). The TSC will then meet with the site PIs and sponsors to determine whether modification to the protocol or study conduct is warranted. The DMEC can, of course, make recommendations on protocol modifications or study continuation to the TSC at any time if they feel that these are needed for any reason. Any outcome affecting the protocol or study conduct will be communicated accordingly with regulatory authorities, ethics committees, investigators and sites.

Missing data will be assessed for any differential 'missingness' between randomised groups and investigated using appropriate missing data mechanisms.

Outcomes

- Primary end point is the percentage change in CTX over the 24-week period of the study.
- Secondary endpoints that will be assessed:
- Percentage change in CTX at week 8.
- Percentage change in TGFβ and P1NP at week 8.
- Percentage change in TGFβ and P1NP over the 24-week period of the study.
- Changes in proxy efficacy outcomes for bone (mass, architecture and strength) using HRpQCT and DXA.
  - Change in DXA LSaBMD and hip.
  - Change in radial and tibial total vBMD by HRpQCT.
- Changes in proxy efficacy outcomes of muscle (strength) using the 'Timed Up and Go' test.
- Change in OI QoL using OI-QOL, a disease-specific tool and a validated generic tool EQ-5D-5L-VAS.

Data management

The main study database will be developed and managed by HHTU (University of Hull, Hull, UK) within the HHTU randomisation system (RCC).

The database will be built and validated according to study-specific requirements with both automated and manual checks to monitor data quality and completeness. Data will be checked according to procedures detailed in the study-specific sponsor-approved data monitoring plan. Study data will be recorded for both study administration and collection of participant data.

All data will be completely anonymised for purposes of analysis and any subsequent reports or publications. Paper Case Report Forms (CRFs) will be created by HHTU as part of the RCC database build, test and release process. The data collected by sites using paper questionnaires will be entered by the site into the RCC specifically developed for this study. If a site used a paper CRF, the data will be entered into RCC and the paper CRF will remain at the recruiting site as source data.

Each site will hold data according to the General Data Protection Regulation Act (2018) and data will be collated in CRFs identified by a unique Subject ID Number. RCC database access will be given by HHTU Data Management team.

RCC Electronic Data Capture (EDC) data will be analysed by the HHTU Statistician at the end of the study reporting. Sites will receive a copy of their locked dataset before the final analysis takes place. RCC EDC data transfer will comply with the General Data Protection Regulation Act (2018).

Access to data

Direct access will be granted to authorised representatives from co-sponsors, host institution and the regulatory authorities to permit study-related monitoring, audits and inspections.

The database will be 'locked' to obtain the final dataset. A copy of the final study dataset and end of study notification will be sent to the co-sponsors before the randomisation list is released by HHTU prior to the statistical analysis. A copy of the final study dataset will be archived by the HHTU and sent to the Chief Investigator(s). Other authorised researchers requesting access to the dataset for further research may apply through the Chief Investigator(s). Applications will be considered in keeping with the publications policy which will be agreed by the Trial Steering Committee.

Patient and public involvement & engagement

The study OI Patient and Public Involvement (PPI) Group informed this study and supported the value to patients of quantifying the net effects (benefits and side-effects) of losartan in OI. The PPI group felt that the intervention and assessments were acceptable.

Patients were involved in all aspects of study monitoring. PPI members also provided advice as Trial Steering Committee members. They will be mentored and trained by the PPI facilitator and supported by the OI PPI group. A study team PPI lead will coordinate and facilitate this activity. PPI group members will be on the Trial Management Group. We are committed to active patient involvement at all stages of the study to ensure the research is grounded and relevant to the experiences of patients, family members and the wider public. As members of the Trial Management Committee, patients will be involved in all practical and strategic decisions about study conduct and management. Lay members will also be involved in the interpretation of analysed findings and dissemination.

ETHICS AND DISSEMINATION

Regulatory approvals and trial oversight

This is a Clinical Trial of an IMP as defined by the EU Directive 2001/20/EC. A Clinical Trial Authorisation (CTA) is required in each EU participating country. This study will recruit in the UK and Europe; each country will submit their study protocol to their own National Competent Authorities.

In the UK, the trial protocol and amendments have been approved by London - London Bridge Research Ethics Committee (REC reference: 23/LO/015) and by the MHRA. In Italy, the study protocol and amendments have been approved by the Italian and European ethics and regulatory authorities (Clinical Trials Information System European Union (CTIS EU) portal according to EU Regulation 536/2014).

Safety considerations and adverse event reporting

In terms of study safety/tolerability outcomes, we are summarising each safety event during the 24-week follow-up period by dose group. Additionally, we will report the number/percentage of patients who cannot achieve the allocated dose (particular for the 50 and 75 mg dose). For safety monitoring purposes, we will periodically report to DMEC at pre-specified timepoints about patient dose calculation algorithm and reasons for no escalation decision (potassium and hypotension). The DMEC will meet at three monthly intervals in order to ensure that there is no emergent safety signal for any specific dose.

Participants are not expected to have a high morbidity or mortality during the study period due to the eligibility criteria requiring participants to have stable disease and be well enough to tolerate losartan treatment. In addition, the study is administering an IMP that has Marketing Authorisation. However, as this is a feasibility study involving dose escalation methodology, all serious adverse event (SAE) events will be reported. There are no SAE exemptions in this study. The SAE reporting period will begin as soon as consent is taken, whereas serious adverse reaction/suspected unexpected serious adverse reaction (SAR/SUSAR) reporting will not start until they have had their first study IMP administration. There are no expected SARs for this study population other than those listed in section 4.8 of the SmPC for 12.5 mg losartan potassium.

Dissemination

The results will be disseminated in peer-reviewed journals, through local OI, orthopaedic and other relevant clinical

networks and at national and international meetings. Patients participating in the study will be sent a summary of the findings, if requested, coordinated at site level.

PROTOCOL VERSION

This paper is based on Protocol Version 3.2 5 March 2025.

CURRENT TRIAL STATUS

The study opened to recruitment on 21 December 2023 and has a 24-month recruitment

window. Recruitment is expected to be extended to June 2026.

Author affiliations
[1]Hull Health Trials Unit, University of Hull, Hull, UK
[2]Sheffield Children's NHS Foundation Trust, Sheffield, UK
[3]IRCCS Istituto Ortopedico Rizzoli, Bologna, Italy
[4]The University of Sheffield, Sheffield, UK

Acknowledgements We especially acknowledge the support of REMEDI4ALL Consortium and are grateful to the members of the Trial Management Group, Data Monitoring and Ethics Committee, Trial Steering Committee and Patient and Public Involvement Group for their ongoing support of the study.

Contributors NB is the guarantor. NJB, JC and LS developed the study design and supported the acquisition of funding. CH provided statistical expertise in the clinical trial design. MHS drafted the manuscript. NJB and JC reviewed and revised the manuscript. KP and CC developed co-sponsorship agreements. All authors contributed to the refinement of the study protocol and to the reviewing and editing of the manuscript.

Funding The REMEDi4ALL project has received funding from the European Union's Horizon Europe Research & Innovation programme under grant agreement No 101057442.

Disclaimer Views and opinions expressed in this publication are, however, those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the granting authority can be held responsible for them.

Competing interests NJB is global CI for the Ultragenyx/Mereo Biopharma sponsored studies (ORBIT and COSMIC) of setrusumab in children and young adults with OI. All other authors have no competing interest to declare.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Consent obtained directly from patient(s).

Provenance and peer review Not commissioned; externally peer reviewed.

ORCID iDs
Mahboobeh Haji Sadeghi https://orcid.org/0000-0002-0332-6933
Judith Cohen https://orcid.org/0000-0002-1538-3521
Laura Simms https://orcid.org/0009-0009-7950-5178
Chao Huang https://orcid.org/0000-0002-3627-7218
Charlotte Heath https://orcid.org/0009-0001-4819-9114
Francesca Gurioli https://orcid.org/0009-0006-4284-8618
Maria Gnoli https://orcid.org/0000-0002-5907-7470
Alice Moroni https://orcid.org/0000-0002-4777-341X
Nick Bishop https://orcid.org/0000-0001-7263-8546

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