THE UNIVERSITY of EDINBURGH

Edinburgh Research Explorer

Prophylactic zoledronic acid therapy to prevent or modify Paget's disease of bone progression in adults with SQSTM1 mutations: the ZiPP RCT

OPEN ACCESS

Prophylactic zoledronic acid therapy to prevent or modify Paget's disease of bone progression in adults with SQSTM1 mutations: the ZiPP RCT

Jonathan Phillips, Deepak Subedi, Steff C Lewis, Catriona Keerie and Stuart H Ralston

Prophylactic zoledronic acid therapy to prevent or modify Paget's disease of bone progression in adults with SQSTM1 mutations: the ZiPP RCT

Jonathan Phillips[1] Deepak Subedi[2] Steff C Lewis[3] Catriona Keerie[3] and Stuart H Ralston[1*]

[1]Centre for Genomic and Experimental Medicine, Institute of Genetics and Cancer, University of Edinburgh, Western General Hospital, Edinburgh, UK
[2]Department of Radiology and Nuclear Medicine, Western General Hospital, Edinburgh, UK
[3]Edinburgh Clinical Trials Unit, Usher Institute, University of Edinburgh, Edinburgh, UK

[*]Corresponding author

Efficacy and Mechanism Evaluation

Criteria for inclusion in the *Efficacy and Mechanism Evaluation* journal
Manuscripts are published in *Efficacy and Mechanism Evaluation* (EME) if (1) they have resulted from work for the EME programme, and (2) they are of a sufficiently high scientific quality as assessed by the reviewers and editors.

EME programme

The Efficacy and Mechanism Evaluation (EME) programme funds ambitious studies evaluating interventions that have the potential to make a step-change in the promotion of health, treatment of disease and improvement of rehabilitation or long-term care. Within these studies, EME supports research to improve the understanding of the mechanisms of both diseases and treatments.

The programme supports translational research into a wide range of new or repurposed interventions. These may include diagnostic or prognostic tests and decision-making tools, therapeutics or psychological treatments, medical devices, and public health initiatives delivered in the NHS.

The EME programme supports clinical trials and studies with other robust designs, which test the efficacy of interventions, and which may use clinical or well-validated surrogate outcomes. It only supports studies in humans and where there is adequate proof of concept. The programme encourages hypothesis-driven mechanistic studies, integrated within the efficacy study, that explore the mechanisms of action of the intervention or the disease, the cause of differing responses, or improve the understanding of adverse effects. It funds similar mechanistic studies linked to studies funded by any NIHR programme.

The EME programme is funded by the Medical Research Council (MRC) and the National Institute for Health and Care Research (NIHR), with contributions from the Chief Scientist Office (CSO) in Scotland and National Institute for Social Care and Health Research (NISCHR) in Wales and the Health and Social Care Research and Development (HSC R&D), Public Health Agency in Northern Ireland.

This article

Abstract

Prophylactic zoledronic acid therapy to prevent or modify Paget's disease of bone progression in adults with SQSTM1 mutations: the ZiPP RCT

Jonathan Phillips[iD],[1] Deepak Subedi[iD],[2] Steff C Lewis[iD],[3]
Catriona Keerie[iD][3] and Stuart H Ralston[iD][1*]

[1]Centre for Genomic and Experimental Medicine, Institute of Genetics and Cancer, University of
  Edinburgh, Western General Hospital, Edinburgh, UK
[2]Department of Radiology and Nuclear Medicine, Western General Hospital, Edinburgh, UK
[3]Edinburgh Clinical Trials Unit, Usher Institute, University of Edinburgh, Edinburgh, UK

*Corresponding author stuart.ralston@ed.ac.uk

Background: Paget's disease of bone is characterised by focal abnormalities of bone turnover resulting in various complications. It often presents at an advanced stage with irreversible bone damage. At this point, the symptomatic benefits of treatment are blunted. Paget's disease of bone has a strong genetic component and the most important susceptibility gene is *SQSTM1*. Carriers of *SQSTM1* mutations have more severe disease with an earlier age of onset than non-carriers and about 80% develop Paget's disease of bone by the seventh decade.

Objectives: The primary objective was to determine if zoledronic acid could prevent new Paget's disease of bone-like bone lesions in *SQSTM1* mutation carriers. Secondary objectives were to assess if zoledronic acid could: modify existing Paget's disease of bone lesions, markers of bone turnover, quality of life, bone pain, anxiety, depression or the risk of complications.

Design: This was a multicentre, double-blind placebo-controlled trial. Genetic screening of the SQSTM1 gene was offered to people with a family history of Paget's disease of bone, identifying 222 mutation carriers who consented to participate. At baseline, a radionuclide bone scan was performed; biochemical markers of bone turnover were measured and questionnaires on pain, quality of life and mental health were completed. Participants completed annual biochemical markers measurements and questionnaires. Adverse events were recorded on a continuous basis. At the end of study, the bone scan was repeated, along with biochemical markers and questionnaires.

Setting: This was a multicentre trial that was conducted at 27 secondary care referral centres for bone disease in 7 countries. All the visits were conducted within a secondary healthcare setting.

Participants Interventions: Participants were randomly allocated to receive a single infusion of the bisphosphonate zoledronic acid 5 mg or an identical placebo.

Main outcome measures: The study's primary outcome measure was defined as the total number of participants who developed new bone lesions on radionuclide bone scans with the characteristics of PDB between the baseline visit and the final end-of-study visit. The secondary outcomes included the number of new PDB bone lesions on radionuclide bone scans, change in the activity of existing PDB bone lesions at the end of study assessed by radionuclide scans; changes in plasma type I collagen C-telopeptides (CTX); plasma procollagen type I amino-terminal propeptide (PINP); serum bone-specific alkaline phosphatase (BAP); quality of life assessed by SF-36, BPI, HADS questionnaires; the presence and severity of localized bone pain assessed by the BPI pain manikin; and the development of

PDB-related skeletal events (PDRSE) in SQSTM1 mutation carriers including new lesions, complications (fractures, deformity), or the need for treatment of PDB.

Methods: This was a multicentre, double-blind placebo-controlled trial. Genetic screening of the *SQSTM1* gene was offered to people with a family history of Paget's disease of bone, identifying 222 mutation carriers who consented to participate. At baseline, a radionuclide bone scan was performed; biochemical markers of bone turnover were measured and questionnaires on pain, quality of life and mental health were completed. Participants were randomly allocated to receive a single infusion of the bisphosphonate zoledronic acid 5 mg or an identical placebo. Participants completed annual biochemical markers measurements and questionnaires. Adverse events were recorded on a continuous basis. At the end of study, the bone scan was repeated, along with biochemical markers and questionnaires.

Results: At baseline, 21/222 individuals (9.5%) had evidence of Paget's disease of bone on bone scans. In the placebo group, 2/90 individuals (2.2%) developed new bone lesions compared with 0/90 (0%) in the zoledronic acid group (odds ratio 0.41, 95% confidence interval 0.00 to 3.43; $p = 0.25$). Eight participants in the placebo group had a poor outcome (new/unchanged/progressing lesions) compared with none in the zoledronic acid group (odds ratio 0.08, 95% confidence interval 0.00 to 0.42; $p = 0.003$). With placebo, 1/29 (3.4%) lesions disappeared compared with 13/15 (86.6%) with zoledronic acid ($p < 0.0001$). One participant allocated to placebo required treatment with zoledronic acid due to a complication of Paget's disease of bone. Significant reductions were observed for serum C-terminal telopeptide ($p < 0.0001$), bone-specific alkaline phosphatase ($p = 0.0003$) and *N*-terminal propeptide of type I procollagen ($p < 0.0001$) in the zoledronic acid group compared with placebo. There was no significant difference between groups in quality of life, pain, anxiety or depression.

Conclusion: Genetic testing for *SQSTM1* mutations coupled with bone scan examination can detect early Paget's disease of bone in those with a family history of the disorder and zoledronic acid treatment can favourably modify its evolution.

The study had some limitations. First, 9.5% of participants already had Paget's disease of bone, reducing power. Second, only two participants developed new lesions compared to the 15% expected. The small number of events meant the study was underpowered for the primary outcome and we were unable to adjust analyses for co-variates or family clustering.

An extended follow-up in the zoledronic acid in the prevention of Paget's disease – long-term extension study is in progress and will provide valuable information on the duration of effects of a single zoledronic acid infusion. It will be important to consider a health economic analysis to model the effects of genetic testing, scanning and zoledronic acid treatment, to evaluate long-term clinical and symptomatic benefits.

Study registration: Current Controlled Trials ISRCTN11616770.

Contents

List of tables

x

List of figures

List of abbreviations

AEs	adverse events	MedDRA	Medical Dictionary for Regulatory Activities
ALP	alkaline phosphatase	MRC	Medical Research Council
ALT	alanine aminotransferase	MSK	musculoskeletal
ANCOVA	analysis of covariance	NTX	N-telopeptide collagen cross links
AST	aspartate aminotransferase		
BPI	Brief Pain Inventory	OR	odds ratio
BAP	bone-specific alkaline phosphatase	PCSS	physical component summary score
CI	confidence interval	PDB	Paget's disease of bone
CTX-I	C-terminal telopeptide of type I collagen	PDRSE	Paget's disease-related skeletal events
eGFR	estimated glomerular filtration rate	PINP	N-terminal propeptide of type I procollagen
FBC	full blood count	REC	Research Ethics Committee
GGT	gamma glutamyl transferase	SAE	serious adverse event
HADS	Hospital Anxiety and Depression Scale	SD	standard deviation
		SF-36	Short Form (36) Health Survey
HTA	Health Technology Assessment	uNTX/Cr	urinary N-telopeptide collagen crosslinks as a ratio to urinary creatinine
HRQoL	health-related quality of life		
IMP	investigational medicinal product	ZA	zoledronic acid
ITT	intention-to-treat	ZiPP	zoledronic acid in the prevention of Paget's disease
MCSS	mental component summary score		

Plain language summary

Paget's disease of bone causes bones to enlarge and become more fragile, potentially leading to pain, deformity, fractures, osteoarthritis and deafness. In normal clinical practice, Paget's disease of bone is often diagnosed at a late stage during the course of the disease when bone damage is irreversible. Early diagnosis and treatment may be beneficial. Mutations in the *SQSTM1* gene can cause Paget's disease of bone to run in families and people with Paget's disease of bone who carry these mutations have more severe and extensive disease with an earlier age at onset. In this study, genetic testing for *SQSTM1* mutations was offered to 1307 people with a family history of Paget's disease of bone with 750 individuals agreeing to be tested. Of these individuals, 350 (46.7%) were found to carry *SQSTM1* mutations and were invited to take part in the trial; 222 people accepted and were enrolled into the study. They were randomly assigned to receive the drug zoledronic acid or a placebo. Both groups were followed for about 7 years. At the start of the study, 9.5% of participants already had Paget's disease of bone lesions. Over time, two people on placebo developed new lesions compared to none on zoledronic acid. In the placebo group, eight people had poor outcomes such as new or worsening lesions versus none in the zoledronic acid group. Treatment with zoledronic acid made existing lesions disappear more often than placebo. One person on placebo required treatment with zoledronic acid as they developed Paget's disease of bone-related symptoms. The study showed that it is feasible to identify people with early Paget's disease of bone by genetic testing coupled with bone scan examination in those with a family history of Paget's disease of bone. Treatment with zoledronic acid caused existing lesions to disappear more often than placebo. Genetic testing for *SQSTM1* mutations coupled with bone scan examination and prophylactic zoledronic acid treatment may be beneficial in people with a family history of Paget's disease of bone.

Scientific summary

Background

In Paget's disease of bone (PDB), the normal process of renewal and repair of the skeleton is abnormal, causing affected bones to enlarge and weaken, resulting in pain, deformity, fractures, secondary osteoarthritis and deafness. People with PDB often present when the disease at an advanced stage with irreversible bone damage. The main treatment option is bisphosphonates, which significantly reduces the increased bone turnover associated with PDB, as well as reducing the associated pain in some patients. However, bisphosphonates cannot reverse bone deformity, deafness or arthritis in PDB with the result that symptomatic benefits are often blunted in people with advanced disease.

The most important susceptibility gene for PDB is *SQSTM1*. Mutations of this gene are observed in up to 40% of individuals with a family history of PDB and up to 15% of those who are unaware of a family history (Makaram NS, Ralston SH. Genetic determinants of Paget's disease of bone. *Curr Osteoporos Rep* 2021;**19**:327–37). Carriers of *SQSTM1* mutations have been shown to have more severe disease with an earlier age of onset than those who do not have such mutations. It has been estimated that about 80% of *SQSTM1* carriers may develop PDB by the time they have reached their seventh decade [Morissette J, Laurin N, Brown JP. Sequestosome 1: mutation frequencies, haplotypes, and phenotypes in familial Paget's disease of bone. *J Bone Miner Res* 2006;**21**(Suppl 2):38–44].

The zoledronic acid (ZA) to prevent the development of Paget's disease [(zoledronic acid in the prevention of Paget's disease (ZiPP)] trial was a double-blind, placebo-controlled randomised study aimed to determine if therapeutic intervention with a single infusion of 5 mg ZA would favourably alter the progression of PDB in people with a family history of PDB who test positive for *SQSTM1* mutations, but who had not yet been diagnosed with PDB.

Objectives

The primary objective was to determine if ZA could prevent the development of bone lesions with the characteristics of PDB in people who carry *SQSTM1* mutations. Additional objectives were to determine if ZA could modify the appearance of existing PDB lesions; modify biochemical markers of bone turnover; modify quality of life, bone pain, anxiety and depression; or modify the risk of complications related to the development of PDB.

Methods

The ZiPP trial was a randomised, double-blind placebo-controlled trial conducted in 25 centres from 7 countries worldwide. A genetic screening programme was offered to 1307 people with a family history of PDB and 750 agreed to be tested. This resulted in the identification of 350 individuals who were carriers of *SQSTM1* mutations but who were not known to have developed PDB. Of these, 222 (63.4%) consented to participate in the study. At the baseline visit, a radionuclide bone scan was performed to detect the presence of bone lesions with the characteristics of PDB; blood samples were taken for analysis of biochemical markers of bone turnover and questionnaires were completed to assess pain, health-related quality of life and anxiety or depression. Participants were then randomly allocated to receive a single infusion of the bisphosphonate ZA 5 mg intravenously or an identical placebo. Both groups were followed up annually where blood samples and questionnaires were repeated. Adverse events (AEs) were recorded on a continuous basis. At the end of study, the bone scan was repeated, bloods were taken for assessment of biochemical markers and questionnaires were repeated.

Results

At baseline, 21/222 individuals (9.5%) already had evidence of PDB on bone scans. Two out of 90 individuals (2.2%) allocated to placebo developed new bone lesions compared with 0 out of 90 (0%) allocated to ZA {odds ratio [OR] = [OR 0.41, 95% confidence interval (CI) 0.00 to 3.43; p = 0.25]}. Eight participants in the placebo group had a poor outcome (lesions that were new, unchanged or progressing) compared with none in the ZA group (OR 0.08, 95% CI 0.00 to 0.42; p = 0.003). In the ZA group, 13/15 lesions present at the start had disappeared compared with 1/29 lesions that disappeared in the placebo group (p < 0.0001, between groups). One participant allocated to placebo required treatment with ZA due to the emergence of symptoms related to PDB. Biochemical markers of bone remodelling were significantly suppressed by ZA. For plasma type I collagen C-terminal telopeptide (CTX), which is a marker of bone resorption, the estimated least squares mean [95% CI] treatment difference taking all timepoints into account was −0.09 [−0.12 to −0.07] (p < 0.0001) in favour of ZA. For plasma procollagen type I amino-terminal propeptide, which is a marker of bone formation, the estimated treatment difference was −16.32 [−22.05 to −10.59] (p < 0.0001) also in favour of the ZA group. Finally, for serum bone-specific alkaline phosphatase (BAP), another marker of bone formation, the estimated treatment effect was −1.68 [−2.59 to −0.78]; p = 0.0003 in favour of ZA. There was no significant difference between the groups in quality of life, bodily pain, or anxiety and depression, and no difference between the groups in AEs or serious adverse events (SAEs).

Limitations

The study had some limitations. First, 9.5% of participants already had Paget's disease, reducing the power to detect treatment effects. Second, only two participants developed new lesions compared to the 15% expected. The small number of events meant that the study was not powered to meet the primary outcome. In addition, the small number of events meant that the study was unable to analyse the data by logistic regression to adjust for covariates or family clustering as was initially planned. This estimate was based on limited cross-sectional data on the increasing PDB incidence with age.

Conclusion

The trial has shown that genetic testing for *SQSTM1* mutations coupled with bone scan examination can detect early PDB in those with a family history of the disorder. It also shows that ZA treatment can favourably modify the evolution of PDB in this participant group. The offer of genetic testing for *SQSTM1* coupled with bone scan examination and targeted intervention with ZA can modify the evolution of PDB in those with a family history of the disorder. Further research is required to evaluate the clinical and health-economic benefits of this approach in the longer term. Further research with an extended follow-up in the ZiPP- long term extension (LTE) study is in progress, and it will provide valuable information on the duration of the effects of a single infusion of ZA on those with existing lesions and the development of new lesions in both treatment groups. Although this was an experimental medicine study, it will now be important to consider a health-economic analysis to try to model the effects of genetic testing, bone scanning and ZA treatment in this participant group to evaluate the likelihood of long-term clinical and symptomatic benefits.

Study registration

Current Controlled Trials ISRCTN11616770.

Funding

Chapter 1　Introduction

Background

Paget's disease of bone (PDB) is a condition associated with abnormalities in the renewal and repair of bone, which has been reported to affect up to 1% of British people over the age of 55 years. The disease is characterised by increased and disorganised bone formation secondary to a focal increase in osteoclastic bone resorption at one or more sites throughout the skeleton. While many patients are asymptomatic, others develop complications such as bone deformity, deafness, pathological fracture and secondary osteoarthritis.[1] Quality of life is adversely affected by these complications resulting in a loss of mobility and independence.[2,3]

Genetic factors are important in PDB, and the disease can be inherited as an autosomal dominant trait in some families.[4–6] Genetic studies have identified 14 genes and/or genomic loci that predispose to PDB and related conditions,[7] but the most important of these is *SQSTM1*, which encodes p62, a scaffold protein in the NFκB signalling pathway.[8–10] Between 20% and 50% of patients with a family history of PDB carry *SQSTM1* mutations and the mutations also occur in between 5% and 20% of patients without a known family history of the disease.[11–17] Individuals with mutations of the *SQSTM1* gene have an earlier age at diagnosis and more clinically severe PDB than those without the mutations.[18] Penetrance has been estimated to be about 80% by the seventh decade.[11,12,14,15,17,19–22] The mutations are highly specific for PDB, and are extremely rare in age and sex-matched controls.[14,15,17,19,23]

Bisphosphonates are regarded as the treatment of choice for PDB. They are highly effective at suppressing biochemical markers of bone turnover and can help in the treatment of bone pain. Various bisphosphonates have been licensed for the treatment of PDB, but the most potent bisphosphonate is zoledronic acid (ZA),[24,25] which can result in a sustained reduction in biochemical markers of bone turnover in more than 95% of subjects with PDB for up to 6.5 years following a single injection.[25,26] The symptomatic benefits of bisphosphonates in people with advanced PDB who already have disease complications such as deformity, deafness and fractures is blunted as these drugs cannot reverse skeletal damage that has already occurred.[27,28]

Objectives

The *primary* objective of the zoledronic acid in the prevention of Paget's disease (ZIPP) trial was to determine if targeted intervention with ZA can prevent the development of new focal bone lesions with the characteristics of PDB in subjects who are genetically predisposed to develop the disease, because they carry pathogenic mutations in *SQSTM1*.

The *secondary* objectives of the trial were to evaluate whether ZA treatment can:

- alter the progression of existing bone lesions in carriers of *SQSTM1* mutations
- decrease or prevent Paget's disease-related skeletal complications in carriers of *SQSTM1* mutations
- reduce or prevent elevated bone turnover carriers of *SQSTM1* mutations
- improve quality of life, bone pain, anxiety and depression in carriers of *SQSTM1* mutations

Chapter 2 Methods

Trial design

Sections of this chapter have been reproduced from Cronin *et al.*[29] and from Philips *et al.* Randomised trial of genetic testing and targeted intervention to prevent the development and progression of Paget's disease of bone. *Ann Rheum* Dis 2024;**83**:529-536. https://doi.org/10.1136/ard-2023-224990 under licence CC BY 4.0.

This study was a multicentre double-blind, placebo-controlled, randomised trial of intravenous ZA or placebo in *SQSTM1* mutation carriers.

The study involved an initial phase of genetic screening to identify eligible participants. Patients with PDB attending outpatient clinics (*n* = 1428) underwent genetic testing for *SQSTM1* mutations using Sanger sequencing of exons 7 and 8 of *SQSTM1* and the intron–exon boundaries using DNA extracted from a venous blood sample according to standard techniques. If the result was positive, 1307 first-degree relatives of these individuals (primarily children) were offered genetic testing for the study. Individuals who consented to undergo testing (*n* = 750) and were found to be positive for *SQSTM1* mutations (*n* = 350) were invited to participate in the interventional phase of the ZiPP study. Two sites in Auckland and Oswestry did not require the participant's parents to be tested since potential participants had already undergone genetic testing for *SQSTM1* as the result of a previous study.

Individuals found to have *SQSTM1* mutations were counselled and randomised to receive either ZA 5 mg or an identical placebo by intravenous infusion. Participants who tested negative for *SQSTM1* mutations were invited to take part in the observational study, which will be described elsewhere. Participants completed a baseline visit, at which point they had safety blood tests, blood and urine tests for biochemical markers of bone metabolism, and had imaging by radionuclide bone scan to look for any evidence of PDB. They were contacted by telephone 1 week after the baseline visit to determine if any adverse effects had occurred following the infusion. Following this, annual visits were carried out when information was collected on medical history, medication, quality of life, pain, anxiety and depression by questionnaires. Blood samples were taken for biochemical markers of bone turnover at each annual visit, and questionnaires were administered to assess quality of life, pain, anxiety and depression. At the end-of-study visit, a radionuclide bone scan and the other assessments performed at the baseline visit were repeated. A summary of the procedures performed at screening and during the study is shown in *Table 1*.

Radionuclide bone scan
Bone lesions were assessed by Technetium-99 radionuclide bone scan, which is recognised to be the most sensitive imaging technique for identifying bone lesions in PDB.[30,31] Participants thought to have PDB-like bone lesions on scan had further imaging performed by X-ray, CT scan or magnetic resonance imaging (MRI) scan if the local investigator considered it clinically indicated. Anonymised bone scans and X-ray images were uploaded to the study database for review. All scans were reviewed by an imaging expert blinded to treatment allocation and were independently reviewed by a second imaging expert, also blinded to treatment allocation, to evaluate the concordance between the observers. The images selected included all of those considered by the primary imaging expert to represent PDB-like lesions. If the experts disagreed on a specific image, it was agreed that a third imaging expert (also blinded to treatment allocation) would be asked to adjudicate but this was not required.

Routine biochemistry
Measurements of serum creatinine, urea and electrolytes, serum total alkaline phosphatase (ALP), serum calcium, albumin and liver function tests – which consisted of aspartate aminotransferase (AST) alanine aminotransferase (ALT), gamma glutamyl transferase (GGT) and bilirubin, along with a full blood count

TABLE 1 Summary of assessments and outcome measures for the ZiPP trial

	Screening visit	Baseline visit	+1 week	Annual review	End of study
Medical history		✓		✓	✓
Current medication		✓		✓	✓
Physical examination		✓			
Height, weight, blood pressure		✓			✓
Routine biochemistry[a]	✓	✓		✓	✓
Routine haematology[b]		✓			✓
Blood for specialised biomarkers[c]		✓		✓	✓
Urine for specialised biomarkers[d]		✓			✓
SQSTM1 genotyping	✓				
25(OH) vitamin D	✓				
Pregnancy test[e]		✓			
Radionuclide bone scan		✓			✓
Radiographs or other imaging[f]		✓			✓
Infusion		✓			
Telephone review			✓		
Food frequency		✓			
SF-36, HADS and BPI[g]		✓		✓	✓
PDRSE[h]				✓	✓

hCG, human chorionic gonadotrophin; *SQSTM1*, sequestosome-1.
a Calcium, albumin and total protein, Alkaline phosphatase, alanine aminotransferase, aspartate aminotranferase, gamma-glutamyl transferase, bilirubin, urea and electrolytes and creatinine.
b FBC.
c Blood samples for measurement of Bone Specific Alkaline Phosphatase, C-terminal telopeptide of type I collagen, and N-terminal propeptide of tyoe I collagen.
d Second-voided morning urine was taken and stored for measurement of *N*-telopeptide collagen cross links, and other specialised markers of.
e A negative pregnancy test was obtained on the day of, or the day before, infusion of the study drug. The preferred method was serum beta-hCG, but a urine beta-hCG is acceptable for centres that are unable to obtain a serum beta-hCG.
f To be taken of relevant areas in subjects suspected to have PDB-like bone lesions on bone scan.
g SF-36 - Short form 36 Health Survey, HADS - Hospital Anxiety and Depression Scale, BPI - Brief Pain Inventory.
h PDRSE - Paget's disease-related skeletal events.

(FBC) – were performed using standard techniques at the local laboratories in participating centres. The estimated glomerular filtration rate (eGFR) was calculated from serum creatinine, gender and weight by the Cockcroft–Gault equation.[32]

Specialised biochemical markers

Specialised biochemical markers of bone turnover that were measured were urine *N*-telopeptide collagen cross links (NTX) corrected for urinary creatinine, C-terminal telopeptide of type I collagen (CTX), bone-specific alkaline phosphatase (BAP) and the *N*-terminal propeptide of type I procollagen (PINP). These measurements were made on fasting samples collected between 09:00 and 12:00 hours, as previous studies have shown that markers of bone resorption have a circadian rhythm and are influenced by food intake.[33] The urine samples were second-voided 'spot' samples collected after an

overnight fast. Markers of bone resorption were urinary NTX and serum CTX-I. These have been found to be elevated in patients with PDB in case–control studies and to correlate with the extent of bone lesions as determined by scintigraphy in PDB[30,34] The markers of bone formation were PINP and BAP since both have been shown to be superior to total ALP at detecting PDB in case–control studies.[30]

Health-related quality of life
At all annual visits, the participants' health-related quality of life (HRQoL) was assessed by the completion of the Short Form (36) Health Survey (SF-36) questionnaire. The SF-36 is a widely used, validated questionnaire[35] previously used to assess quality of life in patients with established PDB.[27,36]

Brief Pain Inventory
The presence and location of pain were assessed by completion of the Brief Pain Inventory (BPI).[37] The BPI was originally developed to evaluate the location and severity of pain in patients with malignant disease, but has since been validated in people with chronic, non-malignant pain.[38] In addition to completing BPI, participants were also asked if they had experienced any pain and bone pain, and to provide information on the site of the pain using a manikin.

Hospital Anxiety and Depression questionnaire
Anxiety and depression were assessed by the Hospital Anxiety and Depression Scale (HADS).[39] This questionnaire was chosen since it was quick and simple to administer, and it has been extensively validated in many countries and settings.[40]

Paget's disease-related skeletal events
Participants were evaluated clinically at the end of study for the presence of Paget's disease-related skeletal events (PDRSE). These included pathological fractures, bone deformity, deafness due to skull involvement and joint replacement surgery or other surgical procedures that are carried out because of PDB. Administration of an antiresorptive drug during the study because of signs or symptoms that are thought to be due to PDB was considered as a PDRSE.

Changes to trial design

In the original protocol, participants in the active treatment arm were to have a second infusion of the study investigational medicinal product (IMP) at 30 months to further suppress bone turnover. However, soon after the ZiPP study had commenced a study by Reid *et al.*[26] showed that a single infusion of ZA could suppress bone turnover in established PDB for at least 6.5 years. This indicated that there was no need to administer a second infusion 30 months after the baseline infusion. The protocol was amended to reflect this change.

The exclusion criteria were updated to be consistent with the ZA summary of product characteristics (SmPC). This involved removing the abnormalities of liver function as exclusion criteria since ZA are not contraindicated in patients with liver disease and can be used without adjustment in patients with abnormal liver function. The exclusion criterion of hypocalcaemia was amended – it was originally an exclusion criterion with a cut-off value of < 2.2 mmol/l. However, due to the different laboratories involved in the study having different reference ranges for serum calcium, it was not viewed as a reliable cut-off value. Therefore, the cut-off value of < 2.2 mmol/l was removed but hypocalcaemia, as defined by the local laboratory reference range, was retained as an exclusion criterion because hypocalcaemia is a contraindication to the use of ZA. The trial was extended by 22 months to 31 May 2022. The trial extension provided additional time for sites to complete interventional final study visits, as the impact of the COVID-19 pandemic interrupted sites from completing end-of-study visits in a timely manner.

Participants

Probands were eligible for genetic testing if they had been diagnosed with PDB and had any relatives who were aged 30 years or older and who had not been diagnosed with PDB. If the proband tested positive for *SQSTM1*, their relatives were offered genetic testing provided they were aged 30 years or older and had not already been diagnosed with PDB. Relatives of probands who tested positive for *SQSTM1* mutations were invited to take part in the trial.

Study setting

This was a multicentre trial that was conducted at 27 secondary care referral centres for bone disease in 7 countries. All the visits were conducted within a secondary healthcare setting. *Table 2* summarises the sites that enrolled participants into the ZiPP trial.

TABLE 2 Summary of assessments and outcome measures for the ZiPP trial

Country	City
UK	Edinburgh
	London – Guy's and St Thomas' Hospital
	Manchester
	Oswestry
	Liverpool
	Bristol
	London – King's College Hospital
	Portsmouth
	Nottingham
Ireland	Dublin
Spain	Barcelona – Hospital Clinic
	Barcelona – Hospital del Mar
	Salamanca
Italy	Turin
	Siena
	Florence
Belgium	Brussels
Australia	Perth
	Geelong
	Bone and Joint Institute, Royal Newcastle Centre
	Rural Clinical School, Toowoomba
	Sydney
	Brisbane
New Zealand	Auckland
	Christchurch

Interventions

The IMP or placebo was given by a single intravenous infusion and comprised of either ZA (Aclasta®) (5 mg in 100 ml ready-to-infuse solution) or an identical looking placebo (0.9% saline). Both were given at a constant infusion rate over not < 15 minutes.

Outcomes

Primary outcome measures

The studies primary outcome measure was defined as the total number of participants who developed new bone lesions on radionuclide bone scans with the characteristics of PDB between the baseline visit and the final end-of-study visit. Imaging experts blinded to treatment allocation assessed the lesions. The definition of a new bone lesion was one that had evidence of involvement of a new bone or part of an existing bone at the end-of-study visit that was not thought to be involved at the baseline visit.

Secondary outcome measures

The secondary outcome measures were:

1. Number of new bone lesions on radionuclide bone scan. A new bone lesion was defined as evidence of involvement of a new bone or part of an existing bone at the end-of-study visit that was not thought to be involved at the baseline visit.
2. Change in activity of existing bone lesions at end of study that were present at the baseline assessed by semiquantitative analysis of radionuclide bone scans.
3. The development of PDRSE in carriers of *SQSTM1* mutations, defined as any one of the following:
   A. Development of new bone lesions (as defined previously thought to be due to PDB on imaging).
   B. Development of complications thought to be due to the development or progression of PDB, including pathological fractures, bone deformity, deafness, joint-replacement surgery or other orthopaedic procedures.
   C. Administration of treatment for PDB with an antiresorptive drug because of the development of signs or symptoms thought to be due to PDB, such as pain localised to an affected site or neurological symptoms.
4. The development of increased bone turnover, as assessed by measurement of biochemical markers of bone resorption [urinary N-telopeptide collagen crosslinks as a ratio to urinary creatinine (uNTX/Cr) and CTX] and bone formation (BAP, PINP). These markers were measured using samples provided at baseline, annual visits and the end-of-study visit.
5. Quality of life, pain, anxiety and depression assessed by the validated SF-36,[35] BPI[38] and HADS questionnaires.[40] These questionnaires were completed at baseline, annual visits and the end-of-study visit.
6. Presence and severity of localised bone pain as assessed by the BPI pain manikin at baseline, annual visits and the end-of-study visit.

Changes to outcomes

During the study, two secondary outcome measures were introduced. One was to conduct a semi-quantitative analysis of bone lesions found on imaging and the second was to add PDRSE as a composite end point as described in subsection 3 of the secondary outcome measures.

Sample size

The sample size was chosen assuming that 15% of patients in the placebo group and 1.5% of patients in the active (ZA) treatment group would develop new PDB-like bone lesions during follow-up. This estimate of progression of lesions in the placebo group was based on previous cross-sectional studies.[21] The effect size of the intervention was based on the observation that ZA has been reported to normalise biochemical markers of bone turnover for up to 6.5 years in 95% of patients with established PDB.[26] With this assumption, 85 subjects in each group would provide 89% power to detect a treatment effect of this magnitude at an alpha of 0.05. Since it is possible that more than one affected subject per family could be enrolled, the sample size was inflated to account for relatedness of individuals. This was done by calculating the mean squared ALP values in patients within families who carried the same mutation (271.3) and the mean squared ALP values between families (619.7), and combining this with the estimated average number of two subjects per family who may be enrolled in the study. This resulted in a design effect factor of 1.39, inflating the required sample size to 118 per group. In addition to this, the sample size was further inflated to account for a 10% rate of participants lost to follow-up resulting in a total sample size of 130 subjects per group or 260 subjects in total. The actual number of subjects randomised to the interventional study by the time recruitment had closed in April 2015 was 222 and to the observational study was 135. The decision to stop recruitment was based on funding and justified by recalculating the design factor based on the actual number of subjects per family that had been enrolled into the study (1.5 on average). The design factor was recalculated to be 1.26.

Interim analyses and stopping guidelines

Not applicable.

Randomisation: sequence generation

Randomisation was performed at the individual level with a treatment allocation in a 1 : 1 ratio. The randomisation algorithm was developed by data programmers from the Edinburgh Clinical Trials Unit and was used to generate the randomisation sequence and allocation concealment. The programme was located on the web-based study database following the collection of baseline details for each participant. The baseline information allowed the system to populate the required minimisation input variables, thereby determining which arm the participant was to be randomised to. Once the participant was enrolled and randomised, treatment code was generated. All treatment codes were supplied by the drug manufacturer and were built in blocks of four. This treatment code was then presented to the local hospital pharmacy and the treatment dispensed.

Randomisation: type

Patients were randomised to either ZA or matched placebo infusion, with a treatment allocation ratio of 1 : 1. The randomisation was minimised according to the type of mutation (missense vs. truncating or frameshift), by gender (male/female); on the basis of whether or not bone lesions suggestive of PDB are present on the baseline bone scan, whether ALP levels are elevated at baseline (yes/no) and by age (years) in increments as follows: 30–40, 41–50, 51–60, 61–70, 71+. A random element was incorporated in which there was a 1 in 10 chance of the determined treatment being reversed.

Randomisation: allocation concealment mechanism

Allocation concealment was assured by the fact that the ZA and placebo were prepacked in identical containers and provided by the manufacturer each with its own unique treatment code. Following randomisation, each participant was assigned a treatment number and received the treatment in the corresponding prepacked bottle from the pharmacy at study centres.

Randomisation: implementation

The programme used to generate the randomisation sequence and allocation concealment was generated by data programmers from the Edinburgh Clinical Trials Unit. The programme for randomisation was loaded onto the web-based interface linked to the study database, where the researcher would enter the participant's information required for the randomisation process. Randomisation occurred after the baseline details for a participant had been collected. Therefore, there was adequate information about the participant to allow the system to populate the required minimisation input variables, including which arm the participant was to be randomised to. Once the participant was enrolled, randomisation occurred, which was blinded to both the research team and the subject. The researcher was given a treatment code, which was provided by the drug manufacturer and was built in blocks of four. This treatment code was then presented to the pharmacy and treatment was dispensed.

Blinding

The participants and investigators were blinded to treatment allocation. The ZA and placebo infusions were identical. Breaking the blind would only be performed where knowledge of the treatment is necessary for further management of the patient and was only performed by contacting the local hospital pharmacy, which had the restricted code break details.

Similarity of interventions

The interventions were 100 ml bottles containing clear liquid with an identical appearance. Both were given by intravenous infusion at a constant infusion rate over not < 15 minutes.

Statistical methods

The principal analysis was based on the intention-to-treat (ITT) principle incorporating all randomised participants, regardless of treatment received. It was originally anticipated that a binary logistic regression analysis would be fitted to compare the number of patients developing new bone lesions between treatment groups. The proposed model included terms for treatment group (ZA vs. placebo) adjusted for the minimisation variables used in the randomisation (type of mutation, gender, presence of bone lesions suggestive of PDB, elevated ALP levels, age – all fitted as fixed effects if appropriate).

Due to small numbers of outcome events that resulted in model non-convergence, it was not possible to adjust for the minimisation variables. Instead, an unadjusted Fisher's exact test was used, modelling the odds of developing new lesions, presenting a median unbiased estimate. A median unbiased estimate and a one-sided p-value are presented for the primary outcome of new lesions.

Similarly, the planned sensitivity analyses relating to missing data were not conducted due to the smaller-than-expected number of lesions occurring in both treatment arms.

Repeated measures analysis of covariance (ANCOVA) was used to analyse specialised markers of bone turnover and quality-of-life questionnaires. This technique makes use of all available data and has the capacity to handle unbalanced data under the assumption of missing at random.

Secondary outcomes
The secondary outcome measures were as follows.

The number of new bone lesions
Like the primary outcome, summaries by treatment group and overall were presented, detailing:

- the number of lesions at baseline
- the number of lesions at the end of the study
- the number of new lesions at the end of the study.

Statistical analyses were by Poisson regression analysis, with the plan to adjust for the minimisation variables and, if required, including an overdispersion parameter to account for wide variability in the data. An offset term would also be included in the model to account for differing lengths of patient follow-up.

The number of outcome events was so small that a maximum-likelihood Poisson regression, either with or without covariate adjustment, was not possible. Therefore, an exact Poisson regression (a small sample alternative) was performed. The effect of randomised treatment was measured by the unadjusted rate ratio [and 95% confidence interval (CI)] for ZA versus placebo.

Change in activity of existing bone lesions that were present at baseline
Change in bone lesion activity was analysed using binary logistic regression where change was categorised as disappeared/decreased/showed no change/increased. For those with no lesion at baseline, developing new bone lesions was seen as a poor outcome. For those with lesions at baseline: lesion increase, the development of additional lesions, or no change in existing lesions was seen as a poor outcome. If data had allowed, the analysis would have been stratified by the baseline status of lesion(s)/no lesion. However, there were insufficient lesions for this stratification to be implemented.

Specialised markers of bone turnover
Results of each biomarker sample were modelled using a repeated measures ANCOVA adjusting for the relevant baseline measure and the minimisation variables. The estimated treatment effect and 95% CI were presented for each outcome. An exception was NTX/creatinine since this was only measured once at the beginning and the end of study.

Quality-of-life questionnaires
The following quality-of-life measures were formally analysed:

- BPI
- the SF-36 physical component score (PCS), mental component score (MCS)
- HADS interference score, severity score, anxiety score, depression score, total score.

A repeated measures ANCOVA adjusting for the relevant baseline quality-of-life measure and the minimisation variables was undertaken. The estimated treatment effect and 95% CI were presented for each outcome.

Bone pain scores (BPI Manikin)
Patients experiencing bone pain were asked to score their pain by location and severity via the BPI manikin with scores ranging from 1 (very mild pain) to 10 (most severe pain).

Pain scores were categorised as mild (1–4), moderate (5–6) and severe (7–10), and were summarised by treatment group and visit (baseline, annual review and end of study), assessing the number of patients experiencing pain, and also the number of incidences of pain.

Additionally, a listing of those patients with lesions at baseline and/or the end of the study who also noted bone pain at the site of the lesion was presented. This was with a view to establishing whether there is a link between location of lesions and severity of pain at that location.

Safety

Adverse events (AEs) were summarised by treatment received, and by seriousness, outcome, causality, expectedness and severity. AEs were also summarised by bodily system category [musculoskeletal (MSK), respiratory, cardiovascular etc.]. (No formal testing, safety population.)

Serious adverse events (SAEs) were summarised and listed in line with AE. (No formal testing, safety population.)

Routine biochemistry results were summarised by treatment and visit (baseline, annual review and end-of-study visit). (No formal testing, ITT population.)

For ALP, a formal analysis of the results was undertaken, using a repeated measures ANCOVA approach. The model adjusted for baseline ALP and the minimisation variables. The estimated treatment effect and 95% CI were presented. (ITT population.)

Details were provided of any patients who became pregnant or who have a partner who became pregnant during the study. (No formal testing, safety population.)

Additional analyses

Not applicable.

Chapter 3 Results

Participant flow (consort) diagram

Losses and exclusions

The number of participants who were lost from the study was 42: 21 in the zoledronate arm and 21 in the placebo arm. A summary of the reason for withdrawals and deaths can be found in *Figure 1*.
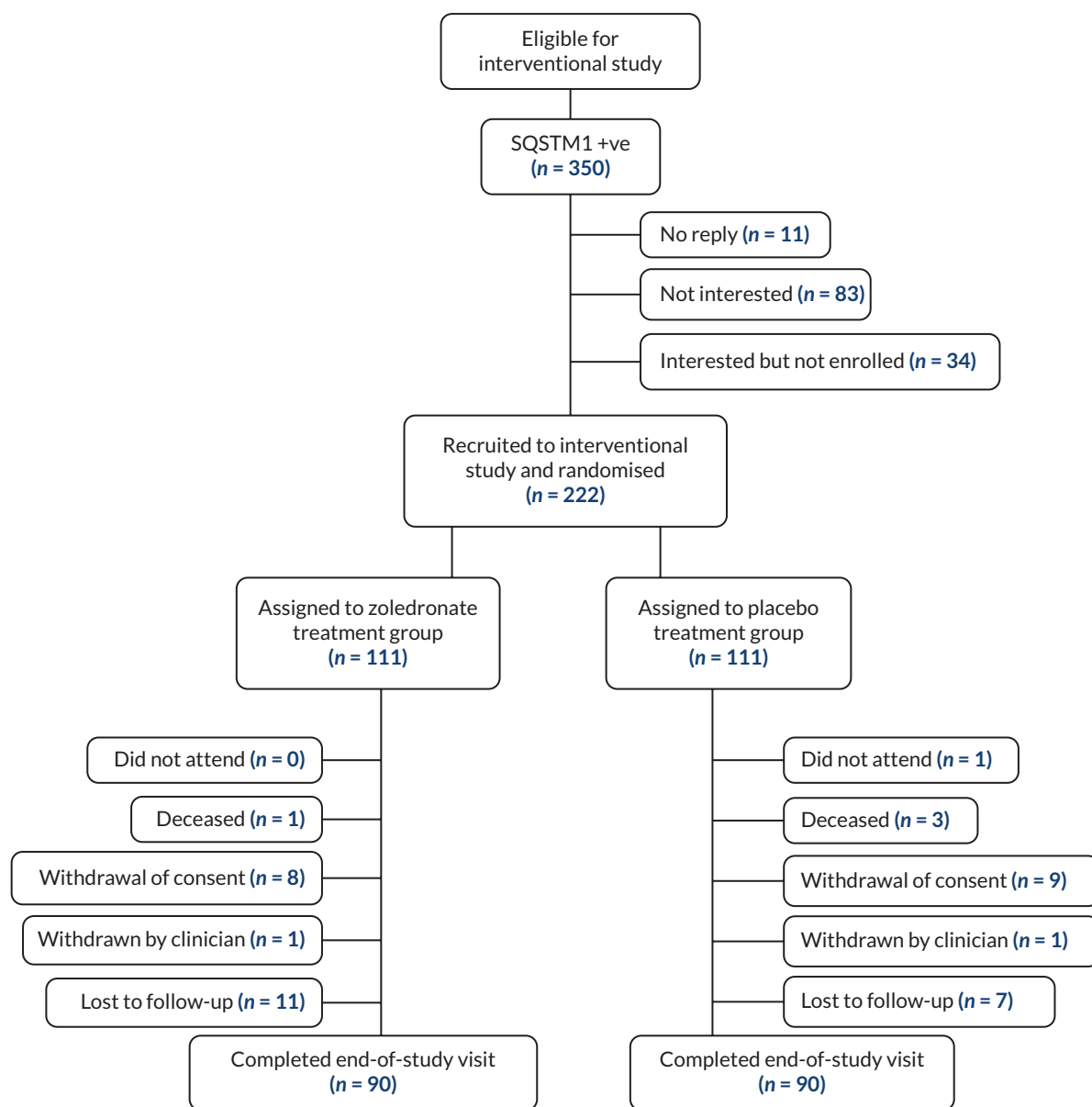
FIGURE 1 Disposition of participants.

Recruitment

The first patient was randomised to the study on 5 March 2010, and the final patient was randomised on 16 April 2015. In total, 222 participants were randomised with 50% (N = 111) being allocated to each treatment group (placebo and zoledronate 5 mg). The recruitment of participants occurred at 25 sites across 7 countries, the distribution of recruitment at each site is shown in *Table 3*.

Recruitment to trial ran from 5 March 2010 to 15th April 2015, when the last participant completed their first visit. Due to the length of time the trial ran for, participants were followed up for a varying length of time, with the mean months of follow-up for the ZA arm being 78.4 [standard deviation (SD) 24.5] and the placebo arm 79.0 (SD 24.3). The mean duration overall in both groups combined was 78.7 (SD 24.0) months. A graphical summary of the duration of follow-up in the trial is shown in *Figure 2*.

TABLE 3 Recruitment by site

Location	Zoledronate 5 mg (*N* = 111)	Placebo (*N* = 111)
Edinburgh	16 (14.4%)	22 (19.8%)
London – Guy's and St Thomas' Hospital	16 (14.4%)	21 (18.9%)
Manchester	12 (10.8%)	15 (13.5%)
Oswestry	3 (2.7%)	1 (0.9%)
Liverpool	6 (5.4%)	6 (5.4%)
Bristol	4 (3.6%)	6 (5.4%)
London – King's College Hospital	1 (0.9%)	0 (0.0%)
Portsmouth	1 (0.9%)	2 (1.8%)
Nottingham	2 (1.8%)	0 (0.0%)
Dublin	6 (5.4%)	4 (3.6%)
Barcelona – Hospital Clinic	5 (4.5%)	4 (3.6%)
Barcelona – Hospital del Mar	0 (0.0%)	1 (0.9%)
Salamanca	4 (3.6%)	4 (3.6%)
Turin	9 (8.1%)	5 (4.5%)
Siena	2 (1.8%)	1 (0.9%)
Florence	1 (0.9%)	1 (0.9%)
Brussels	1 (0.9%)	2 (1.8%)
Perth	6 (5.4%)	2 (1.8%)
Geelong	4 (3.6%)	4 (3.6%)
Royal Newcastle Centre	4 (3.6%)	2 (1.8%)
St Vincent's Hospital, Toowoomba	1 (0.9%)	1 (0.9%)
Sydney	4 (3.6%)	5 (4.5%)
Brisbane	1 (0.9%)	0 (0.0%)
Auckland	1 (0.9%)	2 (1.8%)
Christchurch	1 (0.9%)	0 (0.0%)
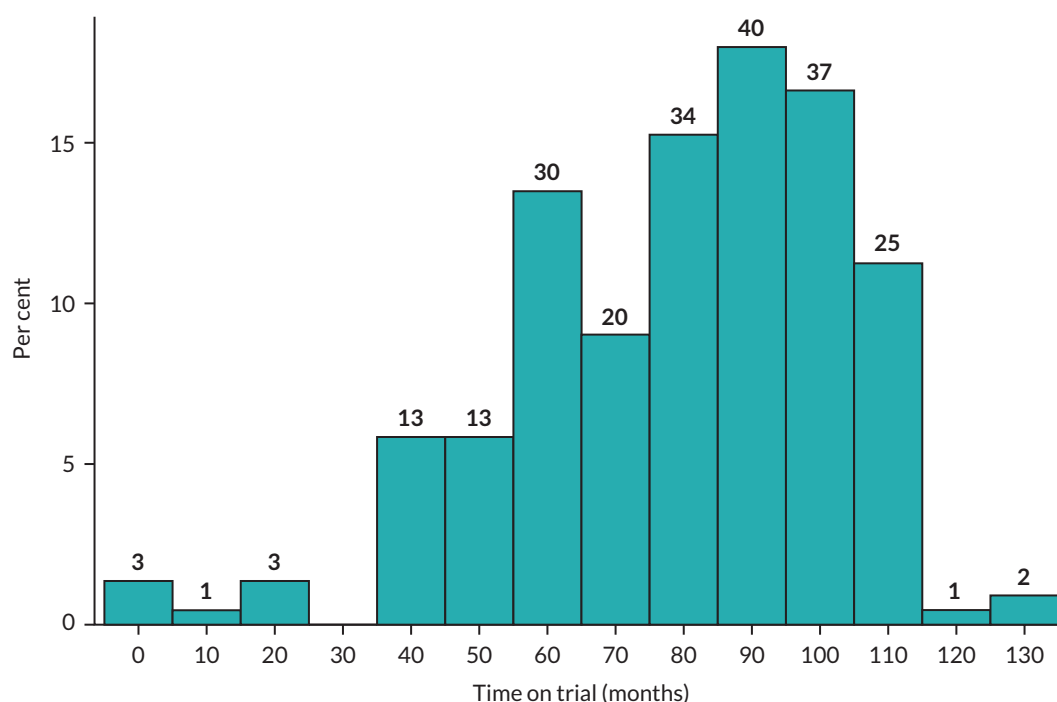
FIGURE 2 Duration of participation in the ZiPP study.

Baseline data

Out of the 222 participants randomised, 101 were male (45.5%), with a mean age of 50.2 years (SD 9.1). Full characteristics of participants in both the ZA and the placebo arm are shown in *Table 4*.

The results of routine biochemistry and haematology at baseline are shown in *Table 5*. Mean values were similar in both treatment groups except for GGT, which was slightly higher in the placebo treatment arm (37.9, SD 50.6) compared with the ZA arm (27.7, SD 17.3).

The percentages of participants in each treatment arm that were either above the upper limit of the reference range for each biochemistry measure is shown in *Table 6*. An exception is serum 25(OH) vitamin D3 where information is provided on the number of individuals deficient, sufficient or normal.

Details of mutations in *SQSTM1* are shown in *Table 7*. The majority of participants (*n* = 202, 91%) had a missense mutation and the remaining 20 had a truncation mutation. The most common missense mutation was 1175C > T resulting in a Pro392Leu amino acid change (P392L).

Previous self-reported fracture history was assessed at baseline as summarised in *Table 8*. In total, 103 (46.4%) out of the 222 participants had fractures at baseline, the most common of which were fractures of wrist (*n* = 31, 14.0%) and other bones (*n* = 31, 14.0%) not categorised in the list. The study did not collect information on the circumstances that led to these fractures occurring.

Numbers analysed

Of the 222 patients enrolled, 180 patients completed the final study visit. In the ZA arm, 90 (81.1%) completed; there were 21 (18.9%) withdrawals: 8 (7.2%) who withdrew consent; 1 (0.9%) who was withdrawn by the clinician; 1 (0.9%) who was deceased; and 11 (9.9%) who were lost to follow-up. In the placebo arm, 90 (81.1%) attended the final visit, with 21 (18.9%) withdrawals: 9 (8.1%) withdrawing consent, 1 (0.9%) withdrawn by clinician, 3 (2.7%) deceased, 7 (6.3%) lost to follow-up; and 1 (0.9%) who

TABLE 4 Baseline characteristics of study population

	Zoledronate (N = 111)	Placebo (N = 111)
Demographics		
Male	50 (45.0%)	51 (45.9%)
Female	61 (55.0%)	60 (54.1%)
Age (years)	49.8 (8.8)	50.5 (9.3)
Relatedness		
Family clusters	60	73
Number of family members	1.4 (0.8)	1.9 (1.2)
Median (range) of family members	1.0 [1–5]	1 [1–7]
Lifestyle		
Current smoker	13 (11.7%)	20 (18.0%)
Previous smoker	45 (40.5%)	55 (49.5%)
Regular drinker	70 (63.1%)	71 (64.0%)
Physical examination		
Weight (kg)	79.5 (17.7)	82.0 (19.6)
Height (cm)	168 (9.0)	169 (9.0)
Body mass index (kg/m^2)	27.9 (5.3)	28.5 (6.3)
Systolic blood pressure (mmHg)	129 (17.0)	130 (15.0)
Diastolic blood pressure (mmHg)	79.6 (13.4)	78.4 (10.5)
Pulse rate (bpm)	70.3 (10.3)	69.7 (11.2)
General appearance		
Normal	109 (98.2%)	109 (98.2%)
Skin		
Normal	99 (89.2%)	104 (93.7%)
Head/neck/ear, nose and throat/eyes		
Normal	106 (95.5%)	108 (97.3%)
Cardiovascular		
Normal	103 (92.8%)	105 (94.6%)
MSK		
Normal	101 (91.0%)	101 (91.0%)
Central nervous system		
Normal	109 (98.2%)	108 (97.3%)
Numbers are N (%), or mean (SD), unless stated		

TABLE 5 Routine biochemistry and haematology in the study population

	Zoledronate (*N* = 111)	Placebo (*N* = 111)
Raised ALP, *N* (%)	4 (3.6%)	4 (3.6%)
ALP (U/l)	78.2 (41.7)	80.1 (53.1)
ALP (adjusted)[a]	0.44 (0.32)	0.47 (0.37)
Calcium (adjusted) (mmol/l)[b]	2.40 (0.11)	2.41 (0.12)
Albumin (g/l)	44.3 (3.6)	44.0 (3.6)
AST (U/l)	24.0 (8.4)	25.1 (11.7)
ALT (U/l)	28.4 (17.1)	27.7 (19.5)
GGT (U/l)	27.7 (17.3)	37.9 (50.6)
Bilirubin (μmol/l)	10.2 (5.7)	10.4 (5.9)
Serum 25(OH) D (nmol/l)	66.7 (46.1)	64.9 (34.1)
Serum creatinine (μmol/l)	72 (13)	74 (13)
Urea (mmol/l)	5.2 (1.3)	5.2 (1.5)
eGFR	86.1 (21.1)	83.3 (17.4)
Routine haematology		
White blood cell count (10^9/l)	6.36 (1.55)	6.21 (1.69)
Haemoglobin (g/l)	141 (13)	142 (13)
Platelets (10^9/l)	243 (57)	240 (63)
Numbers are mean (SD), unless otherwise stated		

a Adjusted results are expressed in relation to the upper limit of normal for the local reference range.
b Adjusted for albumin values.

failed to attend the final visit. Participant 9038801 within the placebo treatment arm attended the final study visit but declined to have an end-of-study bone scan.

Outcomes and estimation

Primary outcome
At baseline, 9 (8.1%) patients in the ZA group were found to have bone lesions typical of PDB, compared with 12 (10.8%) in the placebo group. By the end of the study, only 1 (0.9%) patient had evidence of a bone lesion in the ZA group, compared with 11 (9.9%) in the placebo group.

A summary of participants with bone lesions detected by bone scan at baseline and end of study is provided in *Table 9*.

In the ZA group, none of the participants developed a new bone lesion during the study, while two patients developed new lesions in the placebo group [odds ratio (OR) 0.406, 95% CI 0.000 to 3.425; *p* = 0.246]. The OR of < 1 indicates a treatment effect in favour of zoledronate. One patient with lesions at baseline in the placebo group required rescue therapy with ZA and declined to have a repeat bone scan at the end-of-study assessment.

RESULTS

TABLE 6 Participants with biochemical values above or below the reference ranges

	Zoledronate 5 mg (*N* = 111)	Placebo (*N* = 111)
Serum 25(OH)D3 (nmol/l)		
Deficient (< 25)	10 (9.0%)	10 (9.0%)
Insufficient (25–50)	39 (35.1%)	30 (27.0%)
Normal (> 50)	61 (55.0%)	71 (64.0%)
uNTX/Cr (upper limit = 65)		
Above limit	30 (27.0%)	39 (35.1%)
Below limit	73 (65.8%)	61 (55.0%)
CTX (ng/ml) (upper limit = 0.704 male/1.018 female)		
Above limit	2 (1.8%)	1 (0.9%)
Below limit	101 (91.0%)	100 (90.1%)
BAP (U/l) (upper limit = 42) – baseline visit		
Above limit	1 (0.9%)	1 (0.9%)
Below limit	102 (91.9%)	99 (89.2%)
PINP (ng/ml) (upper limit = 76) – baseline visit		
Above limit	19 (17.1%)	17 (15.3%)
Below limit	84 (75.7%)	84 (75.7%)

Numbers are *N* (%), unless otherwise stated. The numbers do not add up to 100% due to missing values in some individuals

TABLE 7 Summary of *SQSTM1* mutations in the ZiPP study

	Zoledronate (*N* = 111)	Placebo (*N* = 111)
Type of mutation		
Missense	101 (91.0%)	101 (91.0%)
Truncating	10 (9.0%)	10 (9.0%)
Protein coding change		
c.1165 + 1G>A[a]	5 (4.5%)	3 (2.7%)
p.Phe406Val	2 (1.8%)	0 (0.0%)
p.Gly411Ser	7 (6.3%)	2 (1.8%)
p.Gly425Arg	13 (11.7%)	11 (9.9%)
p.Gln371Ter	1 (0.9%)	1 (0.9%)
p.Glu396Ter	1 (0.9%)	3 (2.7%)
p.Ile424Ser	2 (1.8%)	0 (0.0%)
p.Lys378Ter	1 (0.9%)	1 (0.9%)
p.Met404Val	13 (11.7%)	12 (10.8%)
p.Pro392Leu	64 (57.7%)	77 (69.4%)
p.Thr350fs	2 (1.8%)	1 (0.9%)

a This nucleotide change disrupts an intron donor splice site and is predicted to produce a truncated protein at position 390.

TABLE 8 Summary of previous fractures at baseline

	Zoledronate (*N* = 111)	Placebo (*N* = 111)
Fractures	48 (43.2%)	55 (49.5%)
Tibia	6 (5.4%)	6 (5.4%)
Femur	0 (0.0%)	2 (1.8%)
Humerus	1 (0.9%)	5 (4.5%)
Wrist	12 (10.8%)	19 (17.1%)
Clavicle	3 (2.7%)	5 (4.5%)
Ribs	5 (4.5%)	5 (4.5%)
Hand	6 (5.4%)	8 (7.2%)
Foot	11 (9.9%)	8 (7.2%)
Skull	0 (0.0%)	2 (1.8%)
Lumbar spine	2 (1.8%)	1 (0.9%)
Facial bones	3 (2.7%)	6 (5.4%)
Any other bone	15 (13.5%)	16 (14.4%)

Values are number and per cent.

TABLE 9 Participants with bone lesions at baseline and end of study

Patients with lesions	Zoledronate (*N* = 111)	Placebo (*N* = 111)
Baseline		
Yes	9 (8.1%)	12 (10.8%)
No	102 (91.9%)	99 (89.2%)
End of study		
Yes	1 (0.9%)	11 (9.9%)
No	89 (80.2%)	78 (70.3%)
No assessment	21 (18.9%)	22 (19.8%)

Secondary outcomes

Two new PDB lesions developed in patients allocated to placebo compared with no new lesions in the ZA group. There was a highly significant difference between the groups in the appearances of existing lesions as assessed by a semi quantitative analysis of bone scans by imaging experts blinded to treatment allocation. In the ZA group, 13/15 lesions had disappeared (86.7%), 2/15 had decreased (13.3%) and none remained stable or had progressed. In the placebo group, 1/25 had disappeared (3.4%), 12 were thought to have decreased in intensity (41.4%), 8 were thought to be unchanged (27.6%) and 4 had increased in intensity and/or extent (13.8%). None of the participants allocated to ZA had a poor outcome (defined as the development of new lesions, lesions remaining unchanged, or having progressed) compared with eight in the placebo group (OR 0.08, 95% CI 0.00 to 0.42; *p* = 0.003). A summary of the changes in bone lesions that occurred during the trial is presented in *Table 10*.

A summary of change in bone lesions at the individual patient level is summarised in *Table 11*.

TABLE 10 Evolution of bone lesions in the study population

	Zoledronate (*N* = 111)	Placebo (*N* = 111)
Number of lesions at baseline	15	29
Number of lesions at end of study	2	26
Change in activity of existing lesions		
Disappeared	13 (86.7%)	1 (3.4%)
Decreased	2 (13.3%)	12 (41.4%)
No change	0 (0%)	8 (27.6%)
Increased	0 (0%)	4 (13.8%)
No end-of-study assessment	0 (0%)	4 (13.8%)

TABLE 11 Patient-level change in lesion activity

	Zoledronate (*N* = 111)	Placebo (*N* = 111)
No lesion at baseline or end of study	81 (73.0%)	77 (69.4%)
No lesion at baseline; new lesions at end of study[a]	0 (0%)	2 (1.8%)
Lesion(s) at baseline; fewer lesions at end of study or existing lesions decreased	9 (8.1%)	4 (3.6%)
Lesions(s) at baseline; lesions unchanged at end of study	0 (0%)	3 (2.7%)
Lesion(s) at baseline; existing lesions increased in activity at end of study	0 (0%)	3 (2.7%)
No end-of-study assessment	21 (18.9%)	22 (19.8%)

a One participant in the placebo group who required rescue therapy with ZA had four baseline lesions but declined to have an end-of-study bone scan.

The location and outcome of the bone lesions are summarised for the ZA treatment arm and placebo arm in *Tables 12* and *13*, respectively. Note that it was not possible to evaluate patient-level changes in lesion activity in one participant allocated to placebo who received rescue therapy with ZA since they declined to have an end-of-study bone scan. This individual had four lesions at baseline, affecting the left pubic ramus, cervical vertebrae four and five, the ischium and the sacrum. Various skeletal sites were affected with a distribution consistent with PDB and several participants had more than one lesion. As mentioned previously, the most striking finding was the fact that, out of 15 lesions present at baseline in the ZA treatment arm, 13 had disappeared (86.6%), 2 (13.3%) had diminished in activity and no new lesions developed.

Paget's disease-related skeletal events

The PDRSE reported by the local principal investigator are shown in *Table 14*. This identified 3 PDRSEs in the ZA treatment arm compared to 13 in the placebo treatment arm.

On review of these responses at individual participant level it was noted that most participants did not have PDB lesions on scan at either the beginning or end of study, suggesting that there had

TABLE 12 Distribution and evolution of lesions in the ZA group

Skeletal site	Lesions at baseline	Lesion disappeared	Lesion reduced	Lesion stable	Lesion increased	Lesions at end of study
(R) Calcaneus	2	1	1	0	0	1
(L) Femur	2	1	1	0	0	1
(R) Femur	1	1	0	0	0	0
(L) Ilium	3	3	0	0	0	0
(R) Ilium	1	1	0	0	0	0
(L) Ischium	3	3	0	0	0	0
(R) Ischium	1	1	0	0	0	0
L/spine (L1)	1	1	0	0	0	0
L/spine (L4)	1	1	0	0	0	0
Total	15	13	2	0	0	2

TABLE 13 Distribution and evolution of lesions in the placebo group

Skeletal site	Lesions at baseline	Lesion disappeared	Lesion reduced	Lesion stable	Lesion increased	Lesions at end of study
(R) Ilium	2	0	1	0	1	2
C/spine (C2)	1	0	1	0	0	1
(R) Femur	1	0	1	0	0	1
(R) Humerus	1	0	0	1	0	1
(L) Humerus	2	0	0	1	1	2
(L) Ilium	2	0	1	1	0	2
(L) Ischium	3	0	2	1	0	3
(R) Ischium	2	0	1	0	1	2
L/spine (L4)	1	0	1	0	0	2
L/spine (L5)	1	0	0	1	0	1
(L) Radius	1	0	1	0	0	1
Sacrum	1	0	1	0	0	1
T/spine (T12)	3	0	1	1	1	3
T/spine (T2)	2	0	1	1	0	2
T/spine (T7)	1	1	0	0	0	0
T/spine (T9)	1	0	0	1	0	1
Skull (right)	0	0	0	0	0	1
Total	25	1	12	8	4	26

TABLE 14 Summary of PDRSE

	Zoledronate 5 mg (*N* = 111)	Placebo (*N* = 111)
Spinal cord compression	1 (0.9%)	2 (1.8%)
Deafness	1 (0.9%)	7 (6.3%)
Nerve root compression	0 (0%)	2 (1.8%)
Cranial nerve compression	0 (0%)	1 (0.9%)
Bone pain at affected site	1 (0.9%)	1 (0.9%)
Total	3 (2.7%)	13 (11.7%)

Numbers are *N* (%), unless otherwise stated.

been a misunderstanding of the definition of PDRSE at a site level. Because of this, two independent adjudicators were appointed to review the PDRSEs. Following the adjudication, it was concluded that only one participant in the placebo treatment arm had experienced a PDRSE. The PDRSE was nerve root compression presenting with local pain and visualised by imaging at the C3–C5 region in the cervical spine. This participant was given ZA treatment for PDB as rescue therapy.

Specialised biomarkers of bone turnover
Changes in specialised markers of bone turnover are summarised in the graphs in the following sections. Numerical values giving information on means, SDs and numbers of observations at each time point according to treatment group are also provided in *Appendix 1*.

Urinary N-telopeptide as a ratio of creatinine – uNTX/Cr
This analyte is a biochemical marker of bone resorption. Measurements were made at baseline and end of study only. Mean values at baseline and end of study are shown in *Figure 3*, expressed as a ratio to urine creatinine – uNTX/Cr. At baseline, the uNTX/Cr was higher in the ZA treatment arm (89.7 SD 315.6) group compared to the placebo group. (64.7 SD 56.2). When uNTX/Cr was measured at the end of the study, values had decreased in the ZA group to 56.6 (SD 65.3) but increased in the placebo
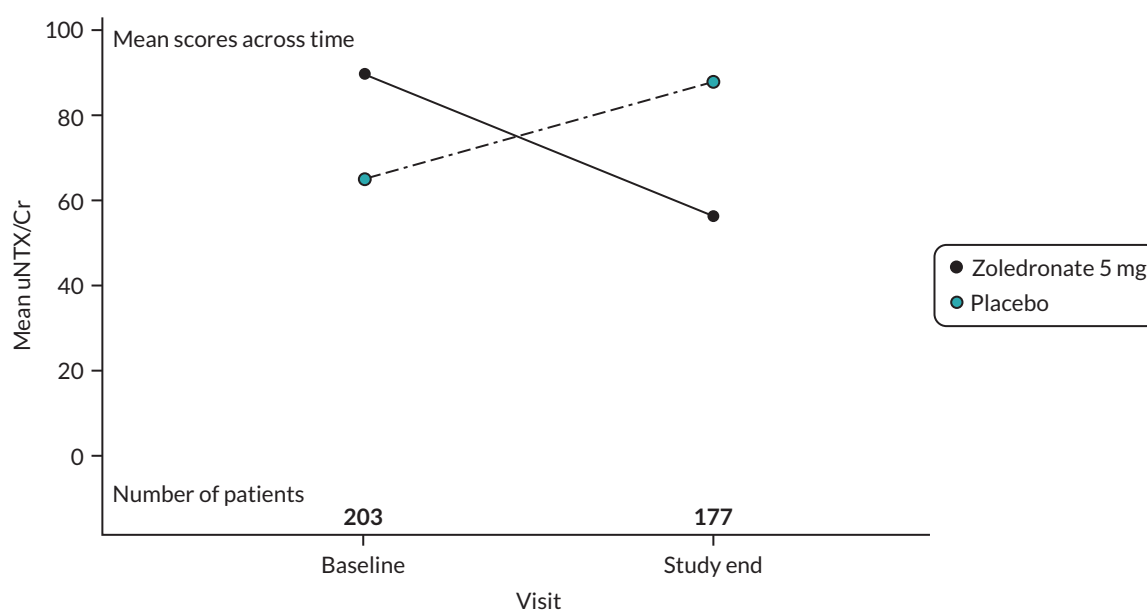
FIGURE 3 Changes in uNTX during the study. Values are means in units of a ratio of uNTX to creatinine – uNTX/Cr.

group, 88.0 (SD 174.8). No formal statistical analysis was conducted for this parameter since results were available only at baseline and study end in contrast to the other three specialised markers of bone marker for which results were available annually up to study end.

Circulating C-terminal telopeptide fragments of collagen

Circulating C-terminal fragments of collagen (CTX) act as a biochemical marker of bone resorption. Changes in CTX are shown in *Figure 4*. Mean baseline levels were similar in the two groups: ZA 0.33 ng/ml (SD 0.17) versus placebo 0.35 ng/ml (SD 0.17). By the end of study, CTX was slightly higher than at baseline in the placebo treatment group (0.41 ng/ml SD 0.20) but had fallen in the ZA group to 0.28 ng/ml (SD 0.14). Overall, there was a significant reduction in CTX in the ZA group as shown in *Figure 4* (−0.09, 95% CI −0.12 to −0.07; *p*-value < 0.0001). The unadjusted mean and SD values for each treatment group and numbers of observations at each timepoint are shown in *Appendix 1*, *Table 17*.

Bone-specific alkaline phosphatase

Circulating concentrations of BAP act as markers of osteoblast activity. Values are shown in *Figure 5*. At baseline, mean values were similar in the two groups (ZA 11.0 U/I SD 7.5 vs. placebo 10.5 U/I SD 8.0). At the end of study, concentrations of BAP had increased in participants treated with ZA (14.1 U/I SD 5.9) and the placebo group (17.2 U/I SD 10.2). Overall, there was a significant reduction in BAP in the ZA group compared with placebo (−1.68, 95% CI −2.59 to −0.78; *p*-value = 0.0003). The unadjusted mean and SD values for each treatment group and numbers of observations at each timepoint are shown in *Appendix 1*, *Table 18*.

Procollagen type 1 N-terminal propeptide

Circulating concentrations of the procollagen type 1 *N*-terminal propeptide act as a marker of bone formation. Values are shown in *Figure 6*. Mean (SD) baseline PINP levels were similar in the two groups (ZA 55.0 ng/ml SD 27.0 vs. placebo 59.5 ng/ml SD 40.8). At the end of study, PINP had fallen in the ZA group (44.0 ng/ml SD 17.4) but increased in the placebo group (63.9 ng/ml SD 67.0). Overall, there was a significant reduction in PINP in the ZA group compared with placebo [−16.32 (−22.05 to −10.59); *p*-value < 0.0001]. The unadjusted mean and SD values for each treatment group and numbers of observations at each timepoint are shown in *Appendix 1*, *Table 19*.
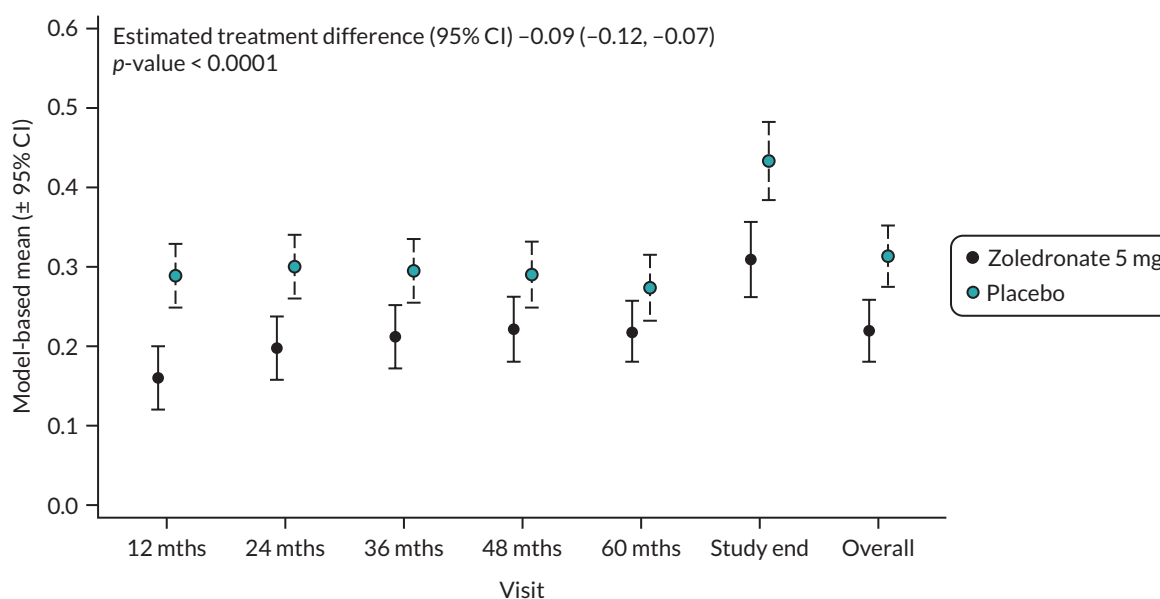
FIGURE 4 Model-based changes in CTX during the study. Values are in ng/ml. Bars are 95% CI. The total number of observations at each of the time points was: baseline – 204, 12 months – 197; 24 months – 188; 36 months – 189; 48 months – 149; 60 months – 112; study end – 178.
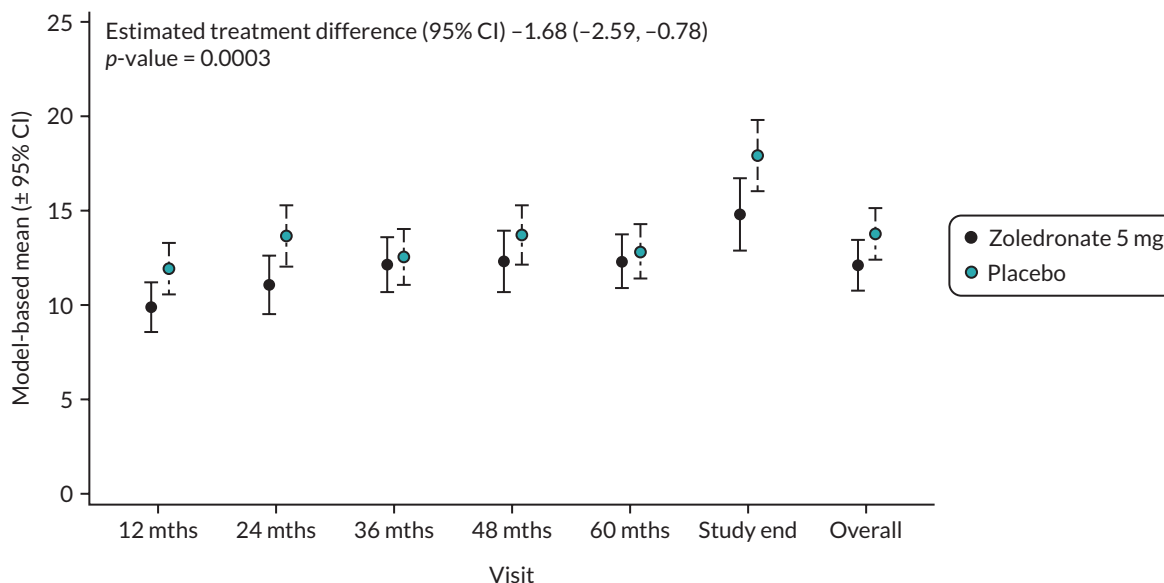
FIGURE 5 Model-based mean changes in BAP during the study. Values are in U/l. Bars are 95% CI. The total number of observations at each of the time points was: baseline – 204, 12 months – 197; 24 months – 188; 36 months – 189; 48 months – 149; 60 months – 112; study end – 178.
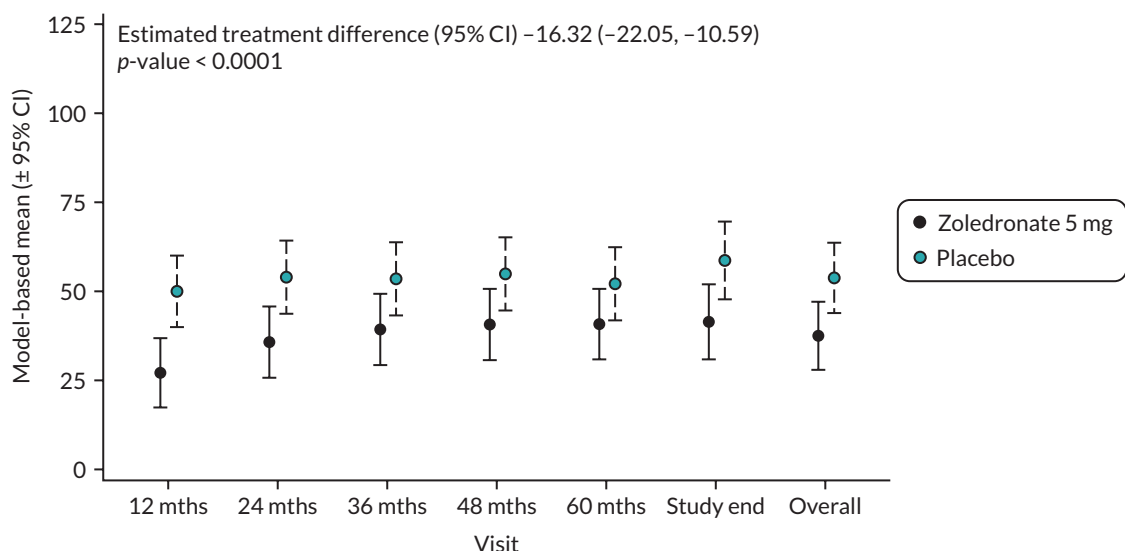
FIGURE 6 Model-based changes in *N*-terminal PINP during the study. Values are in ng/ml. Bars are 95% CI. The total number of observations at each of the time points was: baseline – 204, 12 months – 197; 24 months – 188; 36 months – 189; 48 months – 149; 60 months – 112; study end – 178.

Pain, quality of life, anxiety and depression

Changes in pain, HRQoL and anxiety and depression are summarised in the graphs in the following sections. Numerical values giving information on means, SDs and numbers of observations at each time point according to treatment group are also provided in *Appendix 1*.

Brief Pain Inventory

Pain was assessed using the BPI questionnaire. Two components of pain, interference to life and severity were assessed.

Pain interference score

At baseline, the mean interference score was numerically higher in the ZA group (1.00 SD 1.71) compared to the placebo group (0.82 SD 1.49). During the study interference scores increased with a trend for a lesser increase in the ZA group (*Figure 7*). Overall, there was no significant difference between the groups (−0.37, 95% CI −0.78 to 0.03; *p*-value = 0.070). The unadjusted mean and SD values for each treatment group and numbers of observations at each timepoint are shown in *Appendix 2*, *Table 20*.

Pain severity score

At baseline, the mean BPI severity scores were similar in the two groups: ZA 1.34 SD 1.68 versus 1.24 SD 1.53. During the study, scores in both groups increased but there was no significant difference between the groups (−0.28, 95% CI −0.70 to 0.13; *p*-value = 0.175) (*Figure 8*). The unadjusted mean and SD values for each treatment group and numbers of observations at each timepoint are shown in *Appendix 2*, *Table 21*.

SF-36 quality-of-life questionnaire

The quality of physical and mental components of a participant's life were assessed by the SF-36 questionnaire.

Physical component summary

At baseline, the mean physical component summary scores (PCSS) were similar in the ZA arm 51.4 (SD 8.1) and placebo arm 51.9 (SD 8.6) (see *Figure 9*). By the end of the study, values had fallen slightly in both arms but there was no significant difference between the groups (mean difference, 95% CI) 1.60 (−0.24 to 3.43; *p*-value = 0.086). The unadjusted mean and SD values for each treatment group and numbers of observations at each timepoint are shown in *Appendix 2*, *Table 22*.

### Mental component summary

Values for the SF-36 – mental component summary score (MCSS) were identical at baseline with a mean value of 52.5 (SD 8.5) in both groups (*Figure 10*). During the study, scores tended to increase in the ZA
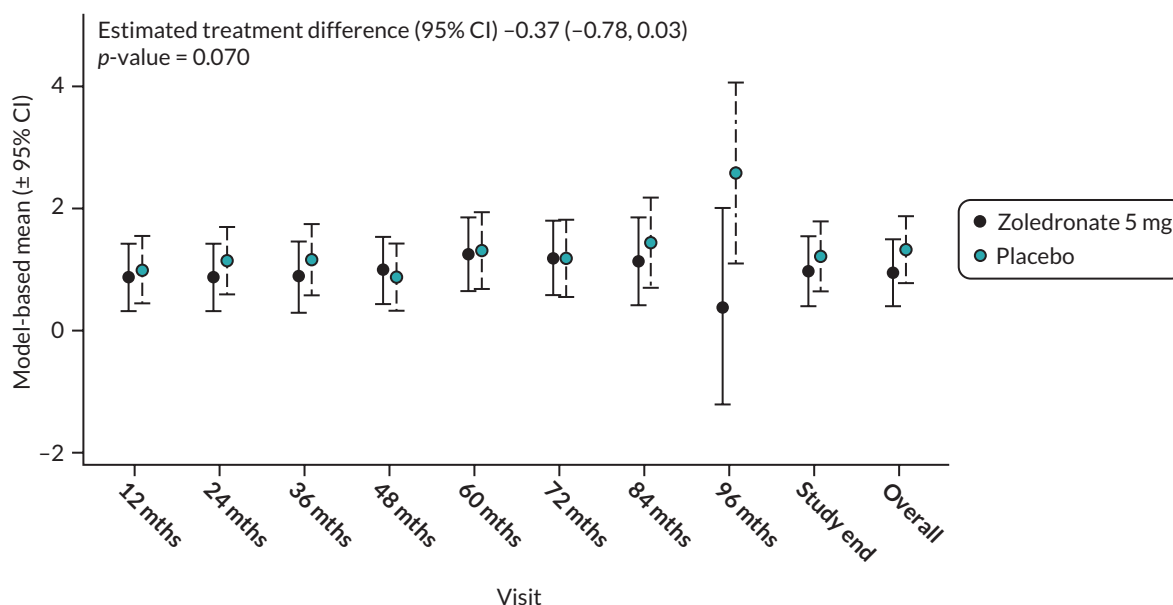
FIGURE 7 Model-based estimates of pain interference score during the study. Scores range between 0 and 10. Higher scores indicate greater pain. Bars are 95% CI. The total number of observations at each of the time points was: baseline – 222, 12 months – 205; 24 months – 198; 36 months – 189; 48 months – 147; 60 months – 113; 72 months – 79; 84 months – 36; 96 months – 11; study end – 177.
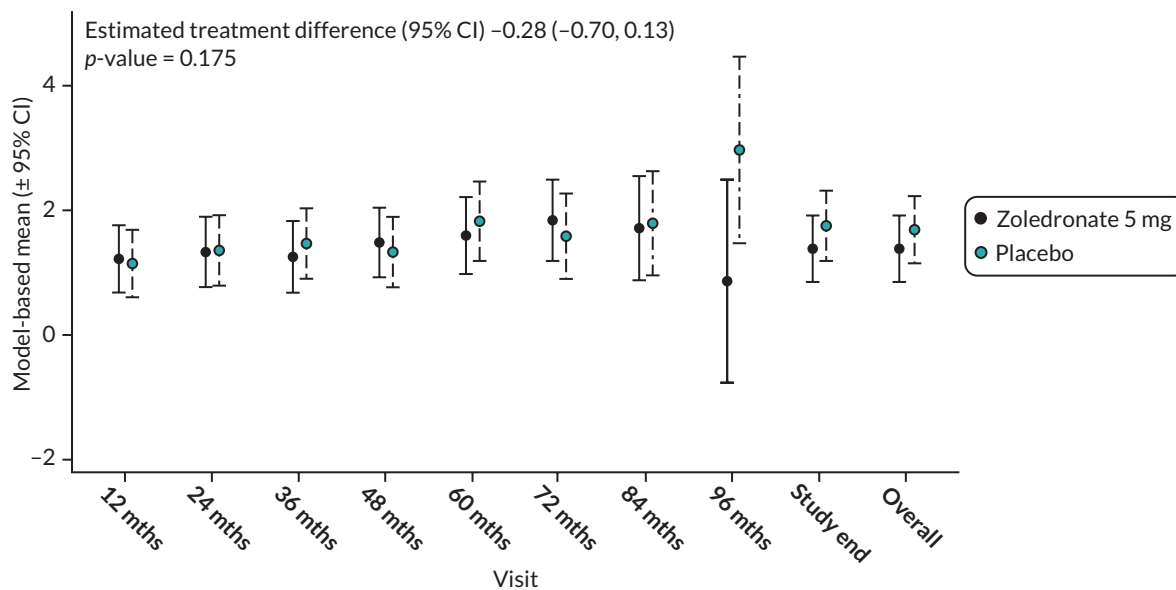
FIGURE 8 Model-based changes in BPI severity score during the study. Scores range between 0 and 10. Higher scores indicate greater pain. Bars are 95% CI. The total number of observations at each of the time points was: baseline – 221, 12 months – 204; 24 months – 199; 36 months – 188; 48 months – 148; 60 months – 112; 72 months – 78; 84 months – 36; 96 months – 11; study end – 176.
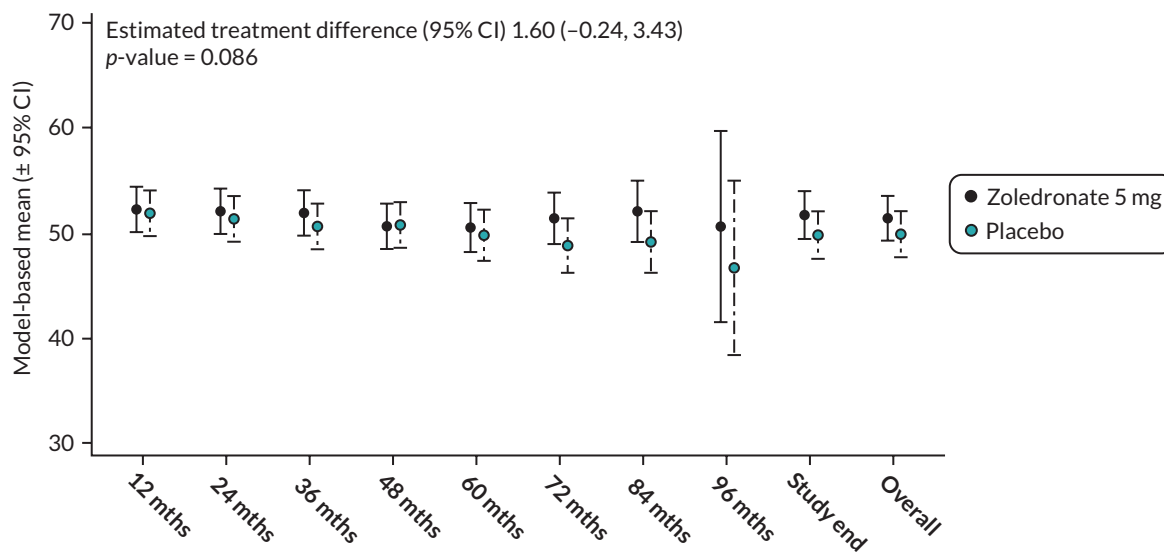

FIGURE 9 Model-based changes in SF-36 physical component summary during the study. A score < 50 indicates health status below average and vice versa. Bars are 95% CI. The total number of observations at each of the time points was: baseline – 222, 12 months – 207; 24 months – 201; 36 months – 187; 48 months – 150; 60 months – 115; 72 months – 81; 84 months – 36; 96 months – 11; study end – 178.

arm but had decreased slightly in the placebo arm. Overall, there was no difference between the groups (mean difference 0.51, 95% CI −1.31 to 2.32; *p*-value = 0.584). The unadjusted mean and SD values for each treatment group and numbers of observations at each timepoint are shown in *Appendix 2*, *Table 23*.

Anxiety and depression
Anxiety and depression were assessed by the HADS.
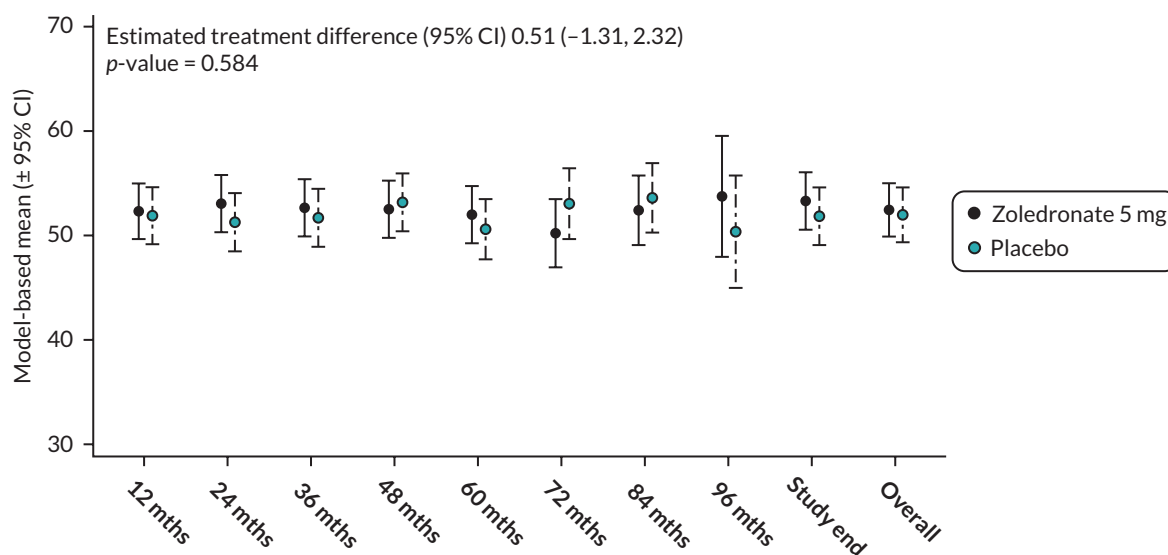
FIGURE 10 Model-based changes in SF-36 mental component summary during the study. A score < 50 indicates health status below average and vice versa. Bars are 95% CI. The total number of observations at each of the time points was: baseline – 222, 12 months – 207; 24 months – 201; 36 months – 187; 48 months – 150; 60 months – 115; 72 months – 81; 84 months – 36; 96 months – 11; study end – 178.

Anxiety

At baseline, there was no significant difference between the groups in levels of anxiety and no difference between groups during the study (*Figure 11*). The unadjusted mean and SD values for each treatment group and numbers of observations at each timepoint are shown in *Appendix 2*, *Table 24*.

Depression

At baseline, mean depression scores were marginally lower in the ZA group compared to the placebo group [3.3 (SD 3.0) vs. 3.5 (SD 2.8)]. As the trial progressed, the ZA treatment group depression score tended to decrease but increased in the placebo group. However, there was no significant difference between the two treatments: mean difference (95% CI) −0.29 (−0.90 to 0.31); *p*-value = 0.340
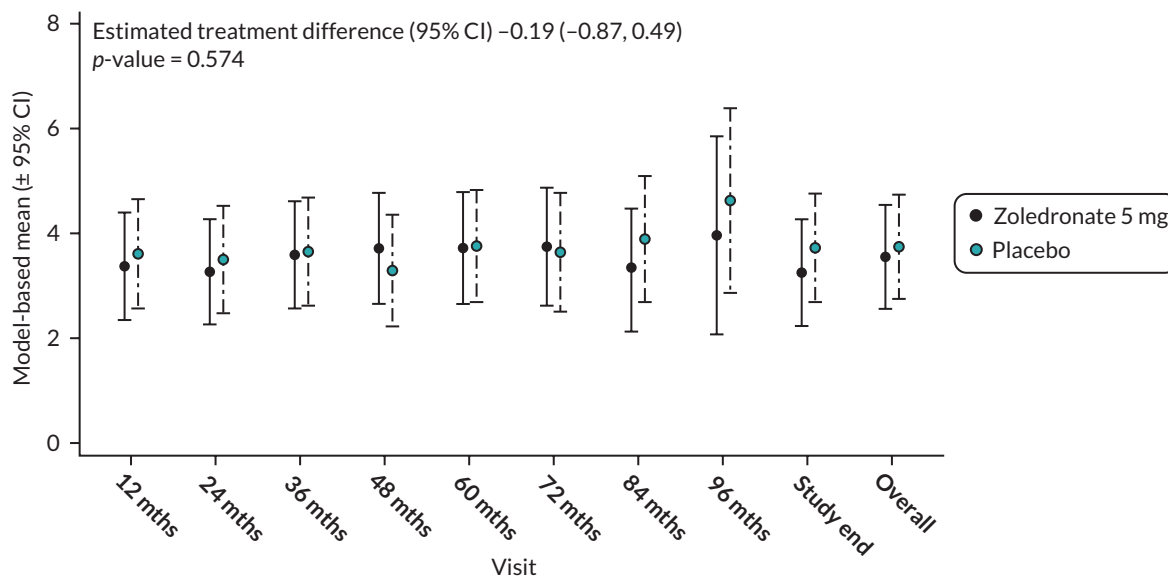
FIGURE 11 Model-based changes in anxiety scores during the study. Scores range between 0 and 10. Higher scores indicate greater levels of anxiety. Bars are 95% CI. The total number of observations at each of the time points was: baseline – 222, 12 months – 207; 24 months – 200; 36 months – 189; 48 months – 150; 60 months – 115; 72 months – 81; 84 months – 36; 96 months – 11; study end – 178.

(*Figure 12*). The unadjusted mean and SD values for each treatment group and numbers of observations at each timepoint are shown in *Appendix 2*, *Table 25*.

Total score – anxiety and depression

There was no significant difference between the two treatments in terms of combined scores for anxiety and depression at baseline or during the study as shown in *Figure 13*. Mean difference −0.48 (95% CI −1.71 to 0.74); *p*-value = 0.437. The unadjusted mean and SD values for each treatment group and numbers of observations at each timepoint are shown in *Appendix 2*, *Table 26*.
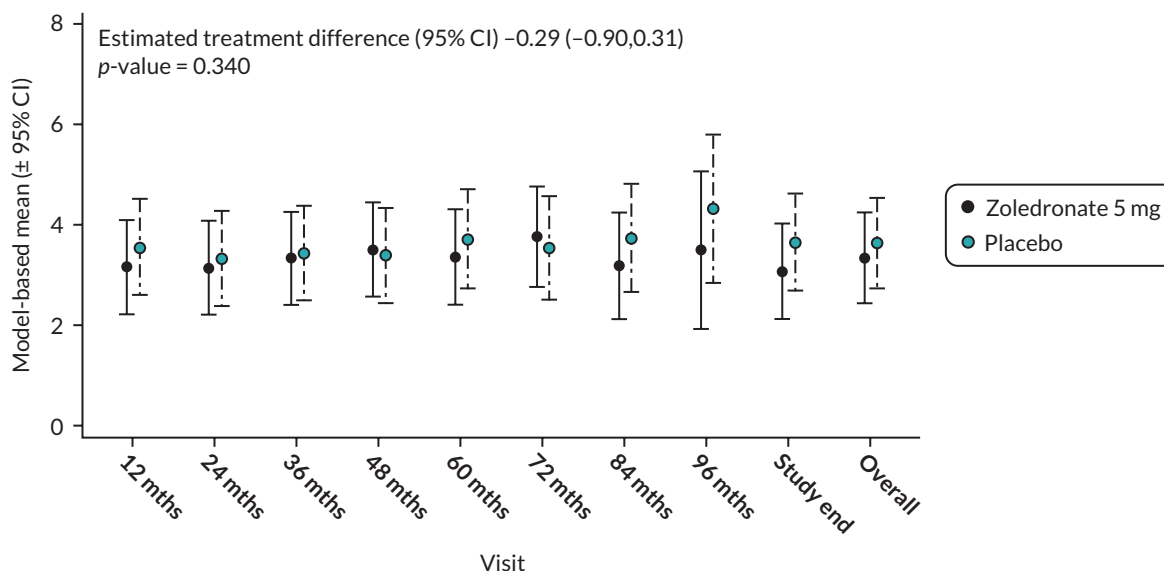
FIGURE 12 Model-based changes in depression scores during the study. Scores range between 0 and 10. Higher scores indicate greater levels of depression. Bars are 95% CI. The total number of observations at each of the time points was: baseline – 222, 12 months – 207; 24 months – 200; 36 months – 189; 48 months – 150; 60 months – 115; 72 months – 81; 84 months – 36; 96 months – 11; study end – 178.
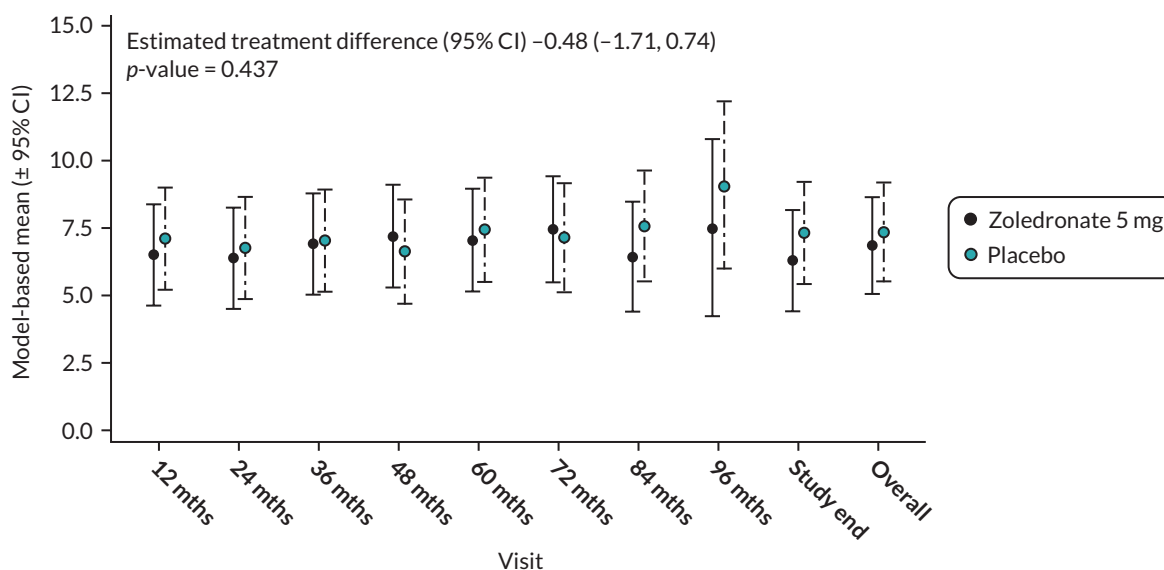
FIGURE 13 Model-based changes in combined anxiety and depression score during the study. Scores range between 0 and 10. Higher scores indicate greater levels of anxiety and depression. Bars are 95% CI. The total number of observations at each of the time points was: baseline – 222, 12 months – 207; 24 months – 200; 36 months – 189; 48 months – 150; 60 months – 115; 72 months – 81; 84 months – 36; 96 months – 11; study end – 178.

Binary outcomes

Not applicable.

Ancillary analyses

Not applicable.

Harms

Adverse events

The proportion of patients experiencing any adverse event (AE) was similar between the ZA and placebo groups: 85 (76.6%) of 111 and 87 (78.4%) of 111, respectively. The total number and type of individual AE according to the MedDRA system organ class (SOC) classification was also similar in both groups (*Table 15*).

TABLE 15 Adverse events in the treatment groups

MedDRA System Organ Class (SOC)	ZA (*N* = 111)	Placebo (*N* = 111)	Total
Total AEs	583	644	1227
Blood and lymphatic system disorders	0 (0.0%)	3 (0.5%)	3 (0.2%)
Cardiac disorders	3 (0.5%)	4 (0.6%)	7 (0.6%)
Congenital, familial and genetic disorders	0 (0.0%)	1 (0.2%)	1 (0.1%)
Ear and labyrinth disorders	6 (1.0%)	9 (1.4%)	15 (1.2%)
Endocrine disorders	4 (0.7%)	3 (0.5%)	7 (0.6%)
Eye disorders	5 (0.9%)	6 (0.9%)	11 (0.9%)
Gastrointestinal disorders	30 (5.1%)	47 (7.3%)	77 (6.3%)
General disorders and administration site conditions	10 (1.7%)	21 (3.3%)	31 (2.5%)
Hepatobiliary disorders	0 (0.0%)	6 (0.9%)	6 (0.5%)
Immune system disorders	2 (0.3%)	1 (0.2%)	3 (0.2%)
Infections and infestations	149 (25.6%)	116 (18.0%)	265 (21.6%)
Injury, poisoning and procedural complications	38 (6.5%)	51 (7.9%)	89 (7.3%)
Investigations	45 (7.7%)	57 (8.9%)	102 (8.3%)
Metabolism and nutrition disorders	8 (1.4%)	11 (1.7%)	19 (1.5%)
MSK and connective tissue disorders	97 (16.6%)	110 (17.1%)	207 (16.9%)
Neoplasms benign, malignant and unspecified (including cysts and polyps)	12 (2.1%)	7 (1.1%)	19 (1.5%)
Nervous system disorders	36 (6.2%)	31 (4.8%)	67 (5.5%)
Pregnancy, puerperium and perinatal conditions	0 (0.0%)	2 (0.3%)	2 (0.2%)

continued

TABLE 15 Adverse events in the treatment groups (*continued*)

MedDRA System Organ Class (SOC)	ZA (*N* = 111)	Placebo (*N* = 111)	Total
Product issues	0 (0.0%)	0 (0.0%)	0 (0.0%)
Psychiatric disorders	10 (1.7%)	17 (2.6%)	27 (2.2%)
Renal and urinary disorders	4 (0.7%)	10 (1.6%)	14 (1.1%)
Reproductive system and breast disorders	14 (2.4%)	16 (2.5%)	30 (2.4%)
Respiratory, thoracic and mediastinal disorders	10 (1.7%)	18 (2.8%)	28 (2.3%)
Skin and subcutaneous tissue disorders	9 (1.5%)	17 (2.6%)	26 (2.1%)
Social circumstances	0 (0.0%)	2 (0.3%)	2 (0.2%)
Surgical and medical procedures	86 (14.8%)	68 (10.6%)	154 (12.6%)
Vascular disorders	5 (0.9%)	10 (1.6%)	15 (1.2%)

Values are number and percentages for the events reported.

The study also analysed AEs that occurred within the first week after the infusion and there was no difference in the number of events between treatment groups (data not shown). There were 8 out of 583 (1.4%) AEs in the ZA-treated group that were judged to be related to the drug, while in the placebo-treated group there were 2 (0.3%) events reported as directly drug-related.

Serious Adverse Eventes

The number of serious adverse events (SAE) in the treatment groups is shown in *Table 16*. The proportion of individuals experiencing SAEs was lower in the ZA group compared with the placebo group: 18/111 (16.2%) versus 25/111 (22.5%) respectively. No SAEs reported were suspected to be drug-related.

Statistical analysis was not conducted to compare the number or type of AEs by study group.

Serious adverse events

See *Table 16*.

TABLE 16 Serious adverse events in the treatment groups

MedDRA System Organ Class (SOC)	Zoledronate 5 mg (N = 111)	Placebo (N = 111)
Total	N = 23	N = 45
Cardiac disorders	3 (13.0%)	3 (6.7%)
Congenital, familial and genetic disorders	0 (0%)	1 (2.2%)
Gastrointestinal disorders	1 (4.4%)	4 (8.9%)
Hepatobiliary disorders	0 (0%)	1 (2.2%)
Infections and infestations	4 (17.4%)	5 (11.1%)
Injury, poisoning and procedural complications	1 (4.4%)	4 (8.9%)
Metabolism and nutrition disorders	0 (0%)	1 (2.2%)
MSK and connective tissue disorders	1 (4.4%)	4 (8.9%)
Neoplasms benign, malignant and unspecified (including cysts and polyps)	2 (8.8%)	3 (6.7%)
Nervous system disorders	6 (26%)	4 (8.9%)
Pregnancy, puerperium and perinatal conditions	0 (0%)	2 (4.4%)
Reproductive system and breast disorders	1 (4.4%)	3 (6.7%)
Surgical and medical procedures	4 (17.4%)	9 (20.0%)
Vascular disorders	0 (0%)	1 (2.2%)

Values are number and percentages for the events reported.

Chapter 4　Discussion

Limitations

There were several limitations to the study. The first was that the study did not anticipate that 9.5% of participants would have already shown bone scan evidence of Paget's disease at the baseline visit. Since the primary end point was the number of participants with new lesions with the characteristics of PDB this reduced the power to detect an effect of treatment. A second limitation was the fact that the proportion of participants developing new lesions was very small; only two participants in the placebo group developed new lesions compared with the 15% expected over a 5-year follow-up when the study was being designed. It should be noted that this assumption was based on historical cross-sectional data and the known increase in occurrence of PDB with increasing age. Up until this study was performed, there has been no prospective study that has looked at the rate of development of PDB with age, with or without treatment. Because of the small number of events, it was not feasible to conduct a logistic regression analysis to look at the influence of baseline characteristics on the development of new lesions or any potential effects of familial clustering. The study also focused on bone scan evidence of PDB lesions as the primary end point, rather than complications of the disease, such as bone pain, pathological fractures or deformity. Although such complications are of clinical importance, it is thought that they occur as the result of uncontrolled active PDB over many decades. It would be very unlikely for an experimental medicine study such as this to detect an effect of ZA treatment on these clinical outcomes over a relatively short period of 5 to 8 years follow-up. Despite this, the study was able to show a significant inhibitory effect of ZA on evolution of existing lesions. The study also observed a numerical decrease in new lesions, albeit not significantly. Since the complications of PDB are thought to arise as the result of uncontrolled increases in disorganised bone remodelling, it is conceivable that the reversal of bone lesions with ZA demonstrated here may translate into more tangible clinical benefits in the long term.

Generalisability

The findings reported here are generalisable to individuals with a family history of Paget's disease who are willing to undergo genetic testing for *SQSTM1* mutations. The study had remarkably good retention of participants when one considers the extended duration of follow-up and the fact that, for many centres, follow-up and closeout of the trial occurred during the COVID-19 pandemic.

Interpretation

Although the primary end point was not met in the study due to the small number of participants with new bone lesions, the study clearly showed that ZA was highly effective at favourably modifying the appearances of existing bone lesions as assessed by bone scan. In the placebo group, only 1/25 lesion disappeared (3.4%), 12/25 were thought to have improved (48.0%); 8/25 were thought to have remained static (32.0%); and 4/25 to have progressed (12.0%). In comparison, 13/15 (86.7%) existing lesions disappeared in the ZA group, 2 (13.3%) improved and none remained static or progressed.

Another important point to emerge from the study was that the intervention with ZA was well tolerated, with an overall balance of AEs and SAEs that was almost identical between the study groups. This also held true when the study looked at the number of AEs which were reported at the telephone review at one week post infusion. The study's conclusion is that it is feasible to offer people with a family history of PDB genetic testing for *SQSTM1* mutations followed by the offer of a radionuclide bone scan in those

who test positive, in the knowledge that this is likely to pick up early disease in about 10% of individuals. It would then be possible to offer these individuals ongoing surveillance or prophylactic treatment with ZA to reduce the risk of the disease progressing with the aim of favourably modify evolution of the disease. It is clinically relevant to consider that the effects of a single infusion of ZA were still apparent in terms of biochemical markers and evidence of lesions on bone scan at a mean of 78.7 months follow-up. Because of this, delivery of this intervention would be eminently feasible in routine clinical practice.

Our study indicates that further research to evaluate the potential clinical and health economic benefits of prophylactic ZA would be indicated in this patient group. While only one patient in the placebo group developed a complication related to PDB, further follow-up of these subjects is in progress in the form of an extension study (ZIPP-long term extension) and it will be interesting to see whether further individuals start to develop symptoms of PDB or complications.

The results of the ZIPP study provide an impetus to consider introducing a programme of genetic testing for *SQSTM1* mutations coupled with bone scan examination in people who have a family history of PDB with the offer of intervention with ZA in those who are found to have bone scan signs of the disease, although it is less clear at the present time whether people who do not have bone scan evidence of PDB would benefit from prophylactic ZA.

Additional information

CRediT contribution statement

Jonathan Philips (https://orcid.org/0009-0006-9027-2668): Project administration, Resources, Visualisation, Writing – original draft preparation, Writing – review and editing. **Deepak Subedi (https://orcid.org/0009-0004-7832-7019):** Data curation, Writing – review and editing. **Steff C Lewis (https://orcid.org/0000-0003-1210-2314):** Formal analysis, Methodology, Software, Writing – review and editing. **Catriona Keerie (https://orcid.org/0000-0001-5459-6178):** Formal analysis, Methodology, Software, Writing – review and editing. **Stuart H Ralston (https://orcid.org/0000-0002-2804-7586):** Conceptualisation, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft preparation, Writing – review and editing.

Acknowledgements

The protocol can be requested from the chief investigator at stuart.ralston@ed.ac.uk or it can be located at https://doi.org/10.1186/ISRCTN11616770

Participating sites

Liverpool **Principal investigator** Lakshminarayan Ranganath Department of Clinical Biochemistry and Metabolic Medicine The University of Liverpool Liverpool L69 3GA United Kingdom Tel: 0151 706 4197 E-mail: lrang@liv.ac.uk	Manchester **Principal investigator** Peter Selby Department of Medicine Manchester Royal Infirmary Manchester M13 9WL United Kingdom Tel: 0161 276 8917 E-mail: peter.selby@mft.nhs.uk
London Guy's & St Thomas' Hospital **Principal investigator** Geeta Hampson Department of Rheumatology St Thomas' Hospital London SE1 7EH United Kingdom Tel: 0207 188 1284 E-mail: geeta.hampson@kcl.ac.uk	Oswestry **Principal investigator** Shu Ho The Robert Jones and Agnes Hunt Orthopaedic and District Hospital Oswestry SY10 7AG United Kingdom Tel: 0169 140 4354 E-mail: shu.ho@nhs.net

London King's College Hospital **Principal investigator** Rama Chandra Clinical Biochemistry King's College Hospital London SE5 9RS United Kingdom Tel: 0203 299 3275 E-mail: rama.chandra@nhs.net	**Portsmouth** **Principal investigator** Steven Young Min Department of Rheumatology Queen Alexandra Hospital Portsmouth PO6 3LY United Kingdom Tel: 0239 228 6199 E-mail: steven.youngmin@porthosp.nhs.uk
Bristol **Principal investigator** Jon Tobias Musculoskeletal Research Unit University of Bristol BS10 5NB United Kingdom Tel: 0239 228 6199 E-mail: jon.tobias@bristol.ac.uk	**Norwich** **Principal investigator** William Fraser Norwich Medical School Faculty of Medicine and Health Sciences University of East Anglia Norwich NR4 7TJ United Kingdom Tel: 0115 969 1169 E-mail: w.fraser@uea.ac.uk
Wrexham **Principal investigator** Mark Garton Betsi Cadwaladr University Health Board/Bwrdd Iechyd Prifysgol Betsi Wrexham Technology Park Wrexham LL13 7YP United Kingdom Tel: 0300 085 8034 E-mail: mark.garton@wales.nhs.uk	**Dublin** **Principal investigators** Malachi McKenna Rachel Crowley Endocrinology Department St. Vincent's University Hospital Dublin 4 Republic of Ireland Tel: +353 1 22 14407 E-mail: malachimckenna@gmail.com; rcrowley@svhg.ie
Norwich **Principal Investigator** Dr Jonathan Tang Norwich Medical School, Faculty of Medicine and Health Sciences, University of East Anglia, Norwich, NR4 7TJ, United Kingdom Tel: 01159 691 169 E-mail: Jonathan.Tang@uea.ac.uk	**Siena** **Principal investigators** Luigi Gennari Rannuccio Nuti Department of Medicine Surgery and Neurosciences University of Sienna Sienna 53100 Italy Tel: +39 577 585 362 E-mail: gennari@unisi.it; nutir@unisi.it
Hospital Clinic, Barcelona **Principal investigator** Nuria Guanabens Department of Rheumatology Hospital Clinic CIBERehd Barcelona 8036 Spain Tel: +34 93 227 5400 E-mail: nguanabens@ub.edu	**Hospital Del Mar, Barcelona** **Principal investigator** Josep Blanch Bone Metabolism Unit Rheumatology Division University Hospital del Mar Barcelona 8003 Spain Tel: +34 93 367 4126 E-mail: jblanch@parcdesalutmar.cat

Salamanca
Principal investigators
Ana Isabel Turrión Nieves
Javier del Pino Montes
Institute for Biomedical Research
University Hospital of Salamanca
Salamanca
37007
Spain
Tel: +34 923 504 376
E-mail: anaturrion@usal.es;
jpino@usal.es

Florence
Principal investigator
Maria Brandi
Department of Internal Medicine University Hospital of Careggi
Florence
50139
Italy
Tel: +39 554 296 586
E-mail: m.brandi@dmi.unifi.it

Turin
Principal investigators
Giancarlo Isaia
Marco Di Stefano
Geriatrics and Metabolic Bone Diseases
AOU San Giovanni Battista di Torino Corso
Torino
10126
Italy
Tel: +39 011 663 7140
E-mail: giancarlo.isaia@unito.it; mdistefano@cittadellasa-lute.to.it

Brussels
Principal investigators
Anne Durnez
Jean-Pierre Devogelaer
Service de Rhumatologie
Clinique Universitaires Saint-Luc
Brussels
B-1200
Belgium
Tel: +32 477 677 606
E-mail: anne.durnez@uclouvain.be;
jean-pierre.devogelaer@uclouvain.be

Sydney Concord
Principal investigator
Markus Seibel
Department of Endocrinology and Metabolism
Concord Repatriation General Hospital
Sydney
New South Wales 2139
Australia
Tel: +61 297 676 109
E-mail: markus.seibel@sydney.edu.au

Geelong
Principal investigator
Mark Kotowicz
Department of Endocrinology and Diabetes
The University of Melbourne
Geelong
3220
Australia
Tel: +61 352 267 764
E-mail: markk@barwonhealth.org.au

Perth
Principal investigator
John Walsh
Department of Endocrinology and Diabetes
Sir Charles Gairdner Hospital
Perth
6009
Australia
Tel: +61 893 462 466
E-mail: John.Walsh@health.wa.gov.au

Brisbane
Principal investigator
Emma Duncan
Endocrinology Department
Royal Brisbane and Women's Hospital
Brisbane
4029
Australia
Tel: +61 736 368 111
E-mail: emma.duncan@qut.edu.au

Toowoomba
Principal investigator
Geoffrey Charles Nicholson
Rural Clinical School, The University of Queensland,
Queensland 4350
Australia
Tel: +61 746 339 702
E-mail: geoff.nicholson49@gmail.com

Auckland
Principal investigator
Anne Horne
Bone & Joint Research Group
Department of Medicine
University of Auckland
Auckland
New Zealand
Tel: +64 99 239 787
E-mail: a.horne@auckland.ac.nz

Newcastle
Principal investigator
Gabor Major
Rheumatology
Bone and Joint Institute
Royal Newcastle Centre
New Lambton Heights
2305
Australia
Tel: +61 249 223 500
E-mail: gabor.major@hnehealth.nsw.gov.au

Christchurch
Principal investigator
Nigel Gilchrist
The CGM Research Trust
Christchurch
8022
New Zealand
Tel: +64 33 377 820
E-mail: nigel.gilchrist@cgm.org.nz

Equality, diversity and inclusion

No restrictions were placed on recruitment to the study based on ethnic background or sex. Recruitment was restricted to individuals > 30 years because Paget's disease increases in incidence with age. It was therefore considered that recruitment of those under the age of 30 years would not be informative. The proportion of females recruited to the study was higher than males (54.5% vs. 45.4%) but this was based on participant choice. All participant information leaflets and documents were available in a selection of languages (English, Italian and Spanish) depending on where the recruitment was carried out. Each of the sites involved used their own staff members, which were representative of suitably qualified individuals from that specific geographic region. The study team was diverse in terms of background and experience, and included clinical support workers, trial managers, research nurses, data managers, statisticians and clinicians with experience of managing Paget's disease. The research uncovered a gap in knowledge about how best to identify individuals with a family history of Paget's disease for further evaluation. The results showed that it may be appropriate to offer people with a family history of Paget's genetic testing for *SQSTM1* mutations coupled with a radionuclide bone scan to detect early disease and the offer of therapeutic intervention with ZA.

Patient data statement

This work uses data provided by patients and collected by the NHS and secondary healthcare institutions around the world. Using patient data is vital to improve health and care for everyone. Patient data should be kept safe and secure to protect everyone's privacy, and it is important that there are safeguards to make sure that they are stored and used responsibly. Everyone should be able to find out about how patient data are used. You can find out more about the background to this citation here: https://understandingpatientdata.org.uk/data-citation

Data-sharing statement

All data requests should be submitted to the corresponding author for consideration. Access to anonymised data may be granted following review.

Ethics statement

The study was reviewed by a Research Ethics Committee (REC). The decision made by the REC is based on the UK Policy Framework for Health and Social Care Research, which sets out principles of good practice in the management and conduct of health and social care research in the UK. These principles protect and promote the interests of patients, service users, and the public in health and social care research, by describing ethical conduct and proportionate, assurance-based management of health and social care research, to support and facilitate high-quality research in the UK that has the confidence of patients, service users and the public.

The study was approved by the Fife and Forth Valley REC on 22 December 2008 (08/S0501/84).

Information Governance statement

The University of Edinburgh is committed to handling all personal information in line with the UK Data Protection Act (2018) and the General Data Protection Regulation (EU GDPR) 2016/679.

Under the Data Protection legislation, University of Edinburgh and NHS Lothian is the Data Controller, and you can find out more about how we handle personal data, including how to exercise your individual rights and the contact details for our Data Protection Officer here.

Disclosure of interests

Completed ICMJE forms for all authors, including all related interests, are available in the toolkit on the NIHR Journals Library report publication page at https://doi.org/10.3310/FTKC2007.

The institution received financial support from the following bodies: Efficacy and Mechanisms Evaluation Programme (NIHR), Versus Arthritis (formerly Arthritis Research UK) European Commission, Wellcome Trust and Medical Research Council (MRC). The Paget's Association provided non-financial support. Novartis provided the IMP. Stuart H Ralston is the Chair of the Paget's Association. Deepak Subedi received funding from GE Healthcare to deliver lectures.

Steff C Lewis was a member of the Health Technology Assessment (HTA) Efficient Study Designs Committee (2015–6) and HTA General Committee (2016–21). Jonathan Phillips declared no conflicts. Catriona Keerie declared no conflicts.

Publications

Cronin O, Forsyth L, Goodman K, Lewis SC, Keerie C, Walker A, *et al.* Zoledronate in the prevention of Paget's (ZiPP): protocol for a randomised trial of genetic testing and targeted zoledronic acid therapy to prevent SQSTM1-mediated Paget's disease of bone. *BMJ Open* 2019;**9**:e030689. https://doi/org/10.1136/bmjopen-2019-030689

Cronin O, Subedi D, Forsyth L, Goodman K, Lewis SC, Keerie C, *et al.* Characteristics of Early Paget's Disease in *SQSTM1* mutation carriers: baseline analysis of the ZiPP study cohort. *J Bone Miner Res* 2020;**35**:1246–52. https://doi.org/10.1002/jbmr.4007. Epub 2020 Apr 20. PMID: 32176830.

Phillips J, Subedi D, Lewis SC, Keerie C, Cronin O, Porteous M, *et al.* Randomised trial of genetic testing and targeted intervention to prevent the development and progression of Paget's disease of bone. *Ann Rheum Dis* 2024;**83**:529-536. https://doi.org/10.1136/ard-2023-224990

References

1. van Staa TP, Selby P, Leufkens HG, Lyles K, Sprafka JM, Cooper C. Incidence and natural history of Paget's disease of bone in England and Wales. *J Bone Miner Res* 2002;**17**(3):465–71. https://doi.org/10.1359/jbmr.2002.17.3.465. PubMed PMID: 11878305.

2. Gold DT, Boisture J, Shipp KM, Pieper CF, Lyles KW. Paget's disease of bone and quality of life. *J Bone Miner Res* 1996;**11**(12):1897–904. https://doi.org/10.1002/jbmr.5650111210. PubMed PMID: 8970891.

3. Langston AL, Campbell MK, Fraser WD, Maclennan G, Selby P, Ralston SH. Clinical determinants of quality of life in Paget's disease of bone. *Calcif Tissue Int* 2007; **80**(1):1–9. https://doi.org/10.1007/s00223-006-0184-2

4. Morales-Piga AA, Rey-Rey JS, Corres-González J, García-Sagredo JM, López-Abente G. Frequency and characteristics of familial aggregation of Paget's disease of bone. *J Bone Miner Res* 1995;**10**(4):663–70. https://doi.org/10.1002/jbmr.5650100421. PubMed PMID: 7610939.

5. Siris ES, Ottman R, Flaster E, Kelsey JL. Familial aggregation of Paget's disease of bone. *J Bone Miner Res* 1991;**6**(5):495–500. https://doi.org/10.1002/jbmr.5650060511. PubMed PMID: 2068956.

6. Seton M, Choi HK, Hansen MF, Sebaldt RJ, Cooper C. Analysis of environmental factors in familial versus sporadic Paget's disease of bone – the New England registry for Paget's disease of bone. *J Bone Miner Res* 2003;**18**(8):1519–24. https://doi.org/10.1359/jbmr.2003.18.8.1519

7. Makaram NS, Ralston SH. Genetic determinants of Paget's disease of bone. *Curr Osteoporos Rep* 2021;**19**:327–37. https://doi.org/10.1007/s11914-021-00676-w

8. Durán A, Serrano M, Leitges M, Flores JM, Picard S, Brown JP, *et al*. The atypical PKC-interacting protein p62 is an important mediator of RANK-activated osteoclastogenesis. *Dev Cell* 2004;**6**(2):303–9. https://doi.org/10.1016/s1534-5807(03)00403-9. PubMed PMID: 14960283.

9. Moscat J, Diaz-Meco MT. The atypical PKC scaffold protein P62 is a novel target for anti-inflammatory and anti-cancer therapies. *Adv Enzyme Regul* 2002;**42**:173–9. https://doi.org/10.1016/s0065-2571(01)00029-2. PubMed PMID: 12123714.

10. Sanz L, Sanchez P, Lallena MJ, Diaz-Meco MT, Moscat J. The interaction of p62 with RIP links the atypical PKCs to NF-kappaB activation. *EMBO J* 1999;**18**(11):3044–53. https://doi.org/10.1093/emboj/18.11.3044. PubMed PMID: 10356400; PMCID: PMC1171386.

11. Hocking LJ, Lucas GJA, Daroszewska A, Mangion J, Olavesen M, Cundy T, *et al*. Domain-specific mutations in Sequestosome 1 (SQSTM1) cause familial and sporadic Paget's disease. *Hum Mol Genet* 2002;**11**(22):2735–9. https://doi.org/10.1093/hmg/11.22.2735

12. Hocking LJ, Lucas GJA, Daroszewska A, Cundy T, Nicholson GC, Donath J, *et al*. Novel UBA domain mutations of SQSTM1 in Paget's disease of bone: genotype phenotype correlation, functional analysis, and structural consequences. *J Bone Miner Res* 2004;**19**(7):1122–7. https://doi.org/10.1359/JBMR.0403015

13. Beyens G, Van Hul E, Van Driessche K, Fransen E, Devogelaer JP, Vanhoenacker F, *et al*. Evaluation of the role of the SQSTM1 gene in sporadic Belgian patients with Paget's disease. *Calcif Tissue Int* 2004;**75**(2):144–52. https://doi.org/10.1007/s00223-004-0244-4

14. Eekhoff EW, Karperien M, Houtsma D, Zwinderman AH, Dragoiescu C, Kneppers ALJ, Papapoulos SE. Familial Paget's disease in The Netherlands: occurrence, identification of new

mutations in the sequestosome 1 gene, and their clinical associations. *Arthritis Rheum* 2004; **50**(5):1650–4. https://doi.org/10.1002/art.20224

15. Johnson-Pais TL, Wisdom JH, Weldon KS, Cody JD, Hansen MF, Singer FR, Leach RJ. Three novel mutations in SQSTM1 identified in familial Paget's disease of bone. *J Bone Miner Res* 2003;**18**(10):1748–53. https://doi.org/10.1359/jbmr.2003.18.10.1748

16. Morissette J, Laurin N, Brown JP. Sequestosome 1: mutation frequencies, haplotypes, and phenotypes in familial Paget's disease of bone. *J Bone Miner Res* 2006;**21**(Suppl 2):38–44. https://doi.org/10.1359/jbmr.06s207

17. Laurin N, Brown JP, Morissette J, Raymond V. Recurrent mutation of the Gene Encoding sequestosome 1 (SQSTM1/p62) in Paget disease of bone. *Am J Hum Genet* 2002;**70**(6):1582–8. https://doi.org/10.1086/340731

18. Visconti MR, Langston AL, Alonso N, Goodman K, Selby PL, Fraser WD, Ralston SH. Mutations of SQSTM1 are associated with severity and clinical outcome in Paget disease of bone. *J Bone Miner Res* 2010;**25**(11):2368–73. https://doi.org/10.1002/jbmr.132

19. Falchetti A, Di Stefano M, Marini F, Del Monte F, Mavilia C, Strigoli D, *et al.* Two novel mutations at exon 8 of *Sequestosome 1* gene (*SQSTM1*) in an Italian series of patients affected by Paget's disease of bone (PDB). *J Bone Miner Res* 2004; **19**(6):1013–7.

20. Collet C, Michou L, Audran M, Chasseigneaux S, Hilliquin P, Bardin T, *et al.* Paget's disease of bone in the French population: novel SQSTM1 mutations, functional analysis, and genotype–phenotype correlations. *J Bone Miner Res* 2007;**22**(2):310–7. https://doi.org/10.1359/jbmr.061106

21. Bolland MJ, Tong PC, Naot D, Callon KE, Wattie DJ, Gamble GD, Cundy T. Delayed development of Paget's disease in offspring inheriting SQSTM1 mutations. *J Bone Miner Res* 2007;**22**(3):411–5. https://doi.org/10.1359/jbmr.061204

22. Rea SL, Walsh JP, Ward L, Yip K, Ward BK, Kent GN, *et al.* A novel mutation (K378X) in the sequestosome 1 gene associated with increased NF-kappaB signaling and Paget's disease of bone with a severe phenotype. *J Bone Miner Res* 2006;**21**(7):1136–45.

23. Lucas GJ, Hocking LJ, Daroszewska A, Cundy T, Nicholson GC, Walsh JP, *et al.* Ubiquitin-associated domain mutations of SQSTM1 in Paget's disease of bone: evidence for a founder effect in patients of British descent. *J Bone Miner Res* 2009;**20**(2):227–31. https://doi.org/10.1359/JBMR.041106

24. Hosking D, Lyles K, Brown JP, Fraser WD, Miller P, Diaz Curiel M, *et al.* Long-term control of bone turnover in Paget's disease with zoledronic acid and risedronate. *J Bone Miner Res* 2007;**22**(1):142–8. https://doi.org/10.1359/jbmr.061001

25. Reid IR, Miller P, Lyles K, Fraser W, Brown JP, Saidi Y, *et al.* Comparison of a single infusion of zoledronic acid with risedronate for Paget's disease. *N Engl J Med* 2005;**353**(9):898–908. https://doi.org/10.1056/NEJMoa044241

26. Reid IR, Lyles K, Su G, Brown JP, Walsh JP, del Pino-Montes J, *et al.* A single infusion of zoledronic acid produces sustained remissions in Paget disease: data to 6.5 years. *J Bone Miner Res* 2011;**26**(9):2261–70. https://doi.org/10.1002/jbmr.438. PubMed PMID: 21638319.

27. Langston AL, Campbell MK, Fraser WD, MacLennan GS, Selby PL, Ralston SH; PRISM Trial Group. Randomized trial of intensive bisphosphonate treatment versus symptomatic management in Paget's disease of bone. *J Bone Miner Res* 2010;**25**(1):20–31. https://doi.org/10.1359/jbmr.090709

28. Tan A, Goodman K, Walker A, Hudson J, MacLennan GS, Selby PL, *et al*. Long-term randomized trial of intensive versus symptomatic management in Paget's disease of bone: the PRISM-EZ study. *J Bone Miner Res* 2017;**32**(6):1165–73. https://doi.org/10.1002/jbmr.3066

29. Cronin O, Forsyth L, Goodman K, Lewis SC, Keerie C, Walker A, *et al*. Zoledronate in the prevention of Paget's (ZiPP): protocol for a randomised trial of genetic testing and targeted zoledronic acid therapy to prevent SQSTM1-mediated Paget's disease of bone. *BMJ Open* 2019;**9**(9):e030689. https://doi.org/10.1136/bmjopen-2019-030689

30. Ralston SH, Corral-Gudino L, Cooper C, Francis RM, Fraser WD, Gennari L, *et al*. Diagnosis and management of Paget's disease of bone in adults: a clinical guideline. *J Bone Miner Res* 2019;**34**(4):579–604. https://doi.org/10.1002/jbmr.3657

31. Fogelman I, Carr D. A comparison of bone scanning and radiology in the assessment of patients with symptomatic Paget's disease. *Eur J Nucl Med* 1980;**5**(5):417–21. https://doi.org/10.1007/BF00261784

32. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron* 1976;**16**(1):31–41. https://doi.org/10.1159/000180580. PubMed PMID: 1244564.

33. Greenblatt MB, Tsai JN, Wein MN. Bone turnover markers in the diagnosis and monitoring of metabolic bone disease. *Clin Chem* 2017;**63**(2):464–74. https://doi.org/10.1373/clinchem.2016.259085

34. Al Nofal AA, Altayar O, BenKhadra K, Qasim Agha OQ, Asi N, Nabhan M, *et al*. Bone turnover markers in Paget's disease of the bone: a systematic review and meta-analysis. *Osteoporosis Int* 2015;**26**(7):1875–91. https://doi.org/10.1007/s00198-015-3095-0

35. Ware JE, Gandek B. Overview of the SF-36 health survey and the International Quality of Life Assessment (IQOLA) project. *J Clin Epidemiol* 1998;**51**(11):903–12. https://doi.org/10.1016/S0895-4356(98)00081-X

36. Tan A, Goodman K, Walker A, Hudson J, MacLennan GS, Selby PL, *et al*.; PRISM-EZ Trial Group. Long-term randomized trial of intensive versus symptomatic management in Paget's disease of bone: the PRISM-EZ study. *J Bone Miner Res* 2017;**32**(6):1165–73. https://doi.org/10.1002/jbmr.3066. Epub 2017/02/09. PubMed PMID: 28176386.

37. Atkinson TM, Mendoza TR, Sit L, Passik S, Scher HI, Cleeland C, Basch E. The Brief Pain Inventory and its 'pain at its worst in the last 24 hours' item: clinical trial endpoint considerations. *Pain Med* 2010;**11**(3):337–46. https://doi.org/10.1111/j.1526-4637.2009.00774.x

38. Tan G, Jensen MP, Thornby JI, Shanti BF. Validation of the Brief Pain Inventory for chronic nonmalignant pain. *J Pain* 2004;**5**(2):133–7. https://doi.org/10.1016/j.jpain.2003.12.005

39. Crawford JR, Henry JD, Crombie C, Taylor EP. Normative data for the HADS from a large non-clinical sample. *Br J Clin Psychol* 2001;**40**(4):429–34. https://doi.org/10.1348/014466501163904. PubMed PMID: 11760618.

40. Stern AF. The Hospital Anxiety and Depression Scale. *Occup Med* 2014;**64**(5):393–4. https://doi.org/10.1093/occmed/kqu024

Appendix 1 Specialised biochemistry

TABLE 17 C-terminal telopeptide (ng/ml)

	Zoledronate 5 mg (N = 111)	Placebo (N = 111)
Baseline (number attended = 222)		
N (missing)	103 (8)	101 (10)
Mean (SD)	0.33 (0.17)	0.35 (0.17)
12 months (number attended = 216)		
N (missing)	100 (8)	97 (11)
Mean (SD)	0.14 (0.07)	0.27 (0.13)
24 months (number attended = 204)		
N (missing)	97 (7)	91 (9)
Mean (SD)	0.18 (0.09)	0.28 (0.17)
36 months (number attended = 198)		
N (missing)	96 (3)	93 (6)
Mean (SD)	0.19 (0.08)	0.28 (0.15)
48 months (number attended = 158)		
N (missing)	75 (6)	74 (3)
Mean (SD)	0.20 (0.07)	0.27 (0.19)
60 months (number attended = 122)		
N (missing)	62 (3)	50 (7)
Mean (SD)	0.20 (0.07)	0.24 (0.09)
Study end (number attended = 180)		
N (missing)	89 (1)	89 (1)
Mean (SD)	0.28 (0.14)	0.41 (0.20)

TABLE 18 Bone-specific alkaline phosphatase (u/l)

	Zoledronate 5 mg (N = 111)	Placebo (N = 111)
Baseline (number attended = 222)		
N (missing)	103 (8)	100 (11)
Mean (SD)	11.0 (7.5)	10.5 (8.0)
12 months (number attended = 216)		
N (missing)	98 (10)	97 (11)
Mean (SD)	8.7 (3.0)	11.0 (3.9)
24 months (number attended = 204)		
N (missing)	97 (7)	91 (9)
Mean (SD)	10.0 (4.3)	12.5 (7.0)

TABLE 18 Bone-specific alkaline phosphatase (u/l) (*continued*)

	Zoledronate 5 mg (*N* = 111)	Placebo (*N* = 111)
36 months (number attended = 198)		
N (missing)	97 (2)	93 (6)
Mean (SD)	11.0 (4.7)	11.4 (3.5)
48 months (number attended = 158)		
N (missing)	74 (7)	75 (2)
Mean (SD)	11.0 (5.4)	12.3 (3.8)
60 months (number attended = 122)		
N (missing)	62 (3)	51 (6)
Mean (SD)	11.2 (3.5)	11.3 (3.8)
Study end (number attended = 180)		
N (missing)	88 (2)	89 (1)
Mean (SD)	14.1 (5.9)	17.2 (10.2)

TABLE 19 Pro-collagen type 1 N-terminal propeptide (ng/ml)

	Zoledronate 5 mg (*N* = 111)	Placebo (*N* = 111)
Baseline (number attended = 222)		
N (missing)	103 (8)	101 (10)
Mean (SD)	55.0 (27.0)	59.5 (40.8)
12 months (number attended = 216)		
N (missing)	100 (8)	97 (11)
Mean (SD)	29.3 (11.0)	53.7 (22.8)
24 months (number attended = 204)		
N (missing)	97 (7)	91 (9)
Mean (SD)	37.8 (16.7)	59.9 (48.1)
36 months (number attended = 198)		
N (missing)	96 (3)	96 (3)
Mean (SD)	40.4 (13.6)	59.3 (42.5)
48 months (number attended = 158)		
N (missing)	75 (6)	74 (3)
Mean (SD)	44.3 (13.6)	61.5 (56.0)
60 months (number attended = 122)		
N (missing)	62 (3)	50 (7)
Mean (SD)	42.9 (12.6)	52.3 (15.3)
Study end (number attended = 180)		
N (missing)	89 (1)	89 (1)
Mean (SD)	44.0 (17.4)	63.9 (67.0)

Appendix 2 Quality-of-life assessments

TABLE 20 Brief Pain Inventory – interference score

	Zoledronate 5 mg (N = 111)	Placebo (N = 111)
Baseline (number attended = 222)		
N (missing)	111 (0)	111 (0)
Mean (SD)	1.00 (1.71)	0.82 (1.49)
12 months (number attended = 216)		
N (missing)	104 (4)	101 (7)
Mean (SD)	1.14 (1.94)	1.17 (2.03)
24 months (number attended = 204)		
N (missing)	101 (3)	97 (3)
Mean (SD)	1.19 (1.93)	1.27 (2.22)
36 months (number attended = 198)		
N (missing)	92 (7)	92 (7)
Mean (SD)	1.25 (2.08)	1.43 (2.30)
48 months (number attended = 158)		
N (missing)	76 (5)	71 (6)
Mean (SD)	1.29 (2.14)	1.19 (2.05)
60 months (number attended = 122)		
N (missing)	61 (4)	52 (5)
Mean (SD)	1.40 (2.17)	1.55 (2.39)
72 months (number attended = 84)		
N (missing)	42 (2)	37 (3)
Mean (SD)	1.37 (2.13)	1.54 (2.27)
84 months (number attended = 37)		
N (missing)	18 (0)	18 (1)
Mean (SD)	1.40 (2.48)	1.79 (2.94)
96 months (number attended = 11)		
N (missing)	5 (0)	6 (0)
Mean (SD)	0.03 (0.06)	2.60 (2.53)
Study end (number attended = 180)		
N (missing)	88 (2)	89 (1)
Mean (SD)	1.26 (2.13)	1.47 (2.26)

BPI questions are scored within a range of 0–10 where higher scores indicate greater pain.

TABLE 21 Brief Pain Inventory – severity score

	Zoledronate 5 mg (*N* = 111)	Placebo (*N* = 111)
Baseline (number attended = 222)		
N (missing)	110 (1)	111 (0)
Mean (SD)	1.34 (1.68)	1.24 (1.53)
12 months (number attended = 216)		
N (missing)	103 (5)	101 (7)
Mean (SD)	1.50 (1.99)	1.41 (1.85)
24 months (number attended = 204)		
N (missing)	103 (1)	96 (4)
Mean (SD)	1.64 (2.08)	1.60 (1.97)
36 months (number attended = 198)		
N (missing)	91 (8)	97 (2)
Mean (SD)	1.58 (1.95)	1.78 (2.10)
48 months (number attended = 158)		
N (missing)	76 (5)	72 (5)
Mean (SD)	1.74 (2.16)	1.71 (2.06)
60 months (number attended = 122)		
N (missing)	60 (5)	52 (5)
Mean (SD)	1.80 (2.22)	2.07 (2.35)
72 months (number attended = 84)		
N (missing)	42 (2)	36 (4)
Mean (SD)	2.15 (2.54)	1.89 (2.03)
84 months (number attended = 37)		
N (missing)	18 (0)	18 (1)
Mean (SD)	1.75 (2.57)	2.13 (2.81)
96 months (number attended = 11)		
N (missing)	5 (0)	6 (0)
Mean (SD)	0.10 (0.22)	2.83 (2.51)
Study end (number attended = 180)		
N (missing)	87 (3)	89 (1)
Mean (SD)	1.66 (1.94)	2.06 (2.12)

BPI questions are scored within a range of 0–10 where higher scores indicate greater pain.

TABLE 22 SF-36 – PCSS

	Zoledronate 5 mg (*N* = 111)	Placebo (*N* = 111)
Baseline (number attended = 222)		
N (missing)	111 (0)	111 (0)
Mean (SD)	51.4 (8.1)	51.9 (8.6)
12 months (number attended = 216)		
N (missing)	105 (3)	102 (6)
Mean (SD)	51.0 (8.4)	50.9 (9.1)
24 months (number attended = 204)		
N (missing)	104 (0)	97 (3)
Mean (SD)	50.9 (9.3)	50.5 (8.4)
36 months (number attended = 198)		
N (missing)	91 (8)	96 (3)
Mean (SD)	50.7 (9.0)	49.4 (9.8)
48 months (number attended = 158)		
N (missing)	77 (4)	73 (4)
Mean (SD)	49.5 (8.8)	49.9 (9.6)
60 months (number attended = 122)		
N (missing)	62 (3)	53 (4)
Mean (SD)	49.6 (9.0)	48.9 (10.6)
72 months (number attended = 84)		
N (missing)	43 (1)	38 (2)
Mean (SD)	50.2 (8.7)	47.6 (11.5)
84 months (number attended = 37)		
N (missing)	18 (0)	18 (1)
Mean (SD)	50.6 (9.5)	47.8 (13.6)
96 months (number attended = 11)		
N (missing)	5 (0)	6 (0)
Mean (SD)	52.8 (4.3)	43.2 (11.9)
Study end (number attended = 180)		
N (missing)	88 (2)	90 (0)
Mean (SD)	50.3 (9.1)	48.6 (9.9)

Each subscale of the SF-36 questionnaire has been normalised with a mean of 50 and a SD of 10. A score < 50 indicates health status below average and vice versa.

TABLE 23 SF-36 – MCSS

	Zoledronate 5 mg (*N* = 111)	Placebo (*N* = 111)
Baseline (number attended = 222)		
N (missing)	111 (0)	111 (0)
Mean (SD)	52.5 (8.5)	52.5 (8.8)
12 months (number attended = 216)		
N (missing)	105 (3)	102 (6)
Mean (SD)	51.9 (8.0)	51.3 (10.2)
24 months (number attended = 204)		
N (missing)	104 (0)	97 (3)
Mean (SD)	52.5 (8.2)	50.9 (9.2)
36 months (number attended = 198)		
N (missing)	91 (8)	96 (3)
Mean (SD)	52.7 (8.6)	50.6 (11.1)
48 months (number attended = 158)		
N (missing)	77 (4)	73 (4)
Mean (SD)	51.7 (10.3)	52.1 (8.7)
60 months (number attended = 122)		
N (missing)	62 (3)	53 (4)
Mean (SD)	52.7 (8.6)	49.7 (10.5)
72 months (number attended = 84)		
N (missing)	43 (1)	38 (2)
Mean (SD)	51.2 (11.3)	51.6 (9.5)
84 months (number attended = 37)		
N (missing)	18 (0)	18 (1)
Mean (SD)	53.8 (8.2)	51.5 (11.2)
96 months (number attended = 11)		
N (missing)	5 (0)	6 (0)
Mean (SD)	57.2 (2.1)	52.8 (9.1)
Study end (number attended = 180)		
N (missing)	88 (2)	90 (0)
Mean (SD)	53.1 (8.2)	50.9 (11.7)

Each subscale of the SF-36 questionnaire has been normalised with a mean of 50 and a SD of 10. A score < 50 indicates health status below average and vice versa.

TABLE 24 Hospital Anxiety and Depression Scale – anxiety score

	Zoledronate 5 mg (N = 111)	Placebo (N = 111)
Baseline (number attended = 222)		
N (missing)	111 (0)	111 (0)
Mean (SD)	3.5 (2.7)	3.7 (3.2)
12 months (number attended = 216)		
N (missing)	105 (3)	102 (6)
Mean (SD)	3.5 (3.2)	3.9 (3.7)
24 months (number attended = 204)		
N (missing)	103 (1)	97 (3)
Mean (SD)	3.4 (3.3)	3.7 (3.1)
36 months (number attended = 198)		
N (missing)	92 (7)	97 (2)
Mean (SD)	3.7 (3.2)	4.2 (3.8)
48 months (number attended = 158)		
N (missing)	77 (4)	73 (4)
Mean (SD)	3.9 (3.7)	3.9 (3.5)
60 months (number attended = 122)		
N (missing)	62 (3)	53 (4)
Mean (SD)	3.3 (3.3)	4.1 (3.5)
72 months (number attended = 84)		
N (missing)	43 (1)	38 (2)
Mean (SD)	3.3 (3.6)	4.2 (3.8)
84 months (number attended = 37)		
N (missing)	18 (0)	18 (1)
Mean (SD)	2.7 (3.2)	4.6 (4.1)
96 months (number attended = 11)		
N (missing)	5 (0)	6 (0)
Mean (SD)	2.4 (1.5)	4.0 (4.0)
Study end (number attended = 180)		
N (missing)	88 (2)	90 (0)
Mean (SD)	3.3 (3.0)	4.1 (3.9)

Anxiety scores range between 0 and 21. Higher scores indicate greater anxiety levels.

TABLE 25 Hospital Anxiety and Depression Scale – depression score

	Zoledronate 5 mg (*N* = 111)	Placebo (*N* = 111)
Baseline (number attended = 222)		
N (missing)	111 (0)	111 (0)
Mean (SD)	3.3 (3.0)	3.5 (2.8)
12 months (number attended = 216)		
N (missing)	105 (3)	102 (6)
Mean (SD)	3.2 (2.7)	3.8 (3.4)
24 months (number attended = 204)		
N (missing)	103 (1)	97 (3)
Mean (SD)	3.2 (2.9)	3.4 (3.0)
36 months (number attended = 198)		
N (missing)	92 (7)	97 (2)
Mean (SD)	3.3 (2.8)	3.7 (3.4)
48 months (number attended = 158)		
N (missing)	77 (4)	73 (4)
Mean (SD)	3.7 (3.5)	3.6 (3.2)
60 months (number attended = 122)		
N (missing)	62 (3)	53 (4)
Mean (SD)	3.0 (2.7)	3.8 (3.4)
72 months (number attended = 84)		
N (missing)	43 (1)	38 (2)
Mean (SD)	3.3 (3.4)	3.5 (3.4)
84 months (number attended = 37)		
N (missing)	18 (0)	18 (1)
Mean (SD)	2.4 (3.2)	3.9 (3.7)
96 months (number attended = 11)		
N (missing)	5 (0)	6 (0)
Mean (SD)	2.2 (2.4)	3.5 (3.3)
Study end (number attended = 180)		
N (missing)	88 (2)	90 (0)
Mean (SD)	3.1 (2.9)	3.9 (3.7)

Depression scores range between 0 and 21. Higher scores indicate greater depression levels.

TABLE 26 Health Anxiety and Depression Scale – total score

	Zoledronate 5 mg (N = 111)	Placebo (N = 111)
Baseline (number attended = 222)		
N (missing)	111 (0)	111 (0)
Mean (SD)	6.9 (5.4)	7.3 (5.6)
12 months (number attended = 216)		
N (missing)	105 (3)	102 (6)
Mean (SD)	6.6 (5.5)	7.7 (6.9)
24 months (number attended = 204)		
N (missing)	103 (1)	97 (3)
Mean (SD)	6.6 (6.0)	7.2 (5.9)
36 months (number attended = 198)		
N (missing)	92 (7)	97 (2)
Mean (SD)	7.0 (5.8)	7.9 (6.9)
48 months (number attended = 158)		
N (missing)	77 (4)	73 (4)
Mean (SD)	7.6 (7.0)	7.5 (6.5)
60 months (number attended = 122)		
N (missing)	62 (3)	53 (4)
Mean (SD)	6.3 (5.8)	7.9 (6.7)
72 months (number attended = 84)		
N (missing)	43 (1)	38 (2)
Mean (SD)	6.6 (6.8)	7.7 (7.0)
84 months (number attended = 37)		
N (missing)	18 (0)	18 (1)
Mean (SD)	5.2 (6.3)	8.6 (7.6)
96 months (number attended = 11)		
N (missing)	5 (0)	6 (0)
Mean (SD)	4.6 (3.8)	7.5 (7.2)
Study end (number attended = 180)		
N (missing)	88 (2)	90 (0)
Mean (SD)	6.4 (5.7)	8.0 (7.3)

Total scores range from 0 to 42. Higher scores indicate greater anxiety and depression levels.