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ZiPP: Randomised Trial of Genetic Testing and Targeted Zoledronic acid Therapy to Prevent SQSTM1 Mediated Paget's Disease

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Funder	Arthritis Research UK Medical Research Council
Funding Reference Number	18163 (Arthritis Research UK) 85281 (Medical Research Council)
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EudraCT Number	2008-005667-34
CTA Number	01384/0211/001-0001
REC Number	08/S0501/84
ISRCTN Number	11616770
Version Number and Date	V1 23Dec2022

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FINAL REPORT DATE: DD/MM/YYYY
STUDY START DATE: 12/01/2009
STUDY END DATE: 31/05/2022
IMP: Zoledronic acid (Aclasta®) 5mg solution for infusion.

1.Title and Abstract

1a. ZiPP: Randomised Trial of Genetic Testing and Targeted Zoledronic acid Therapy to Prevent SQSTM1 Mediated Paget's Disease

1b Abstract

Background
Paget's disease of bone (PDB) is characterised by increased and disorganised bone remodelling. Bisphosphonates are the treatment of choice for pain associated with PDB but have limited effectiveness in those with advanced disease and complications such as bone deformity, secondary osteoarthritis and deafness. Administration of bisphosphonates at an earlier stage in disease evolution may have more favourable effects. Mutations of the *SQSTM1* gene are associated with inheritance of PDB in families. This study sought to determine whether a programme of genetic testing for *SQSTM1* mutations coupled with targeted intervention with the potent bisphosphonate zoledronic acid (ZA) could modify the development or progression of PDB in those with a family history of the disease.

Objectives
To determine if prophylactic therapy with the bisphosphonate zoledronic acid (ZA) can prevent the development of PDB or modify its progression in people with a family history of PDB who carry *SQSTM1* mutations.

Methods and analysis:
Individuals with a family history of PDB aged >30 years who tested positive for *SQSTM1* mutations were eligible to take part. At the baseline visit, they were screened for the presence of bone lesions by radionuclide bone scan. Blood and urine samples were taken for analysis of biochemical markers of bone turnover and for routine biochemistry and haematology. Questionnaires were completed to assess pain, health-related quality of life (HRQoL) anxiety and depression. Participants were randomised to receive a single intravenous infusion of 5 mg ZA or placebo and followed up annually thereafter. Baseline assessments were repeated at the end of study visits. The primary endpoint was the number of participants with new bone lesions on radionuclide bone scan, evaluated by assessors blinded to treatment allocation. Secondary endpoints included changes in appearance of existing bone lesions, biochemical markers of bone turnover, pain, HRQoL, anxiety, depression, and PDB-related skeletal events.

Results
We recruited 222 individuals of whom 111 were randomised to ZA and 111 to placebo. A total of 180 individuals (80.6%) completed the study after a median of 84 months (range 0-127). At baseline, 21/222 individuals (9.5%) had radionuclide bone scan evidence of PDB. Two participants in the placebo group developed new lesions versus none in the ZA group (OR 0.41, 95% CI 0.00 to 3.43, p=0.25). Eight participants in the placebo group had a poor outcome

(lesions which were new, unchanged, or progressing) compared with none in the ZA group (OR =0.08, 95% CI 0.00-0.42, 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 rescue therapy with ZA because of a PDRSE. Significant reductions were observed for serum CTX (p<0.0001), adjusted ALP (p=0.032), bone specific ALP (p=0.0003) and PINP (p<0.0001) in the ZA group. The number and type of adverse events and serious adverse events did not differ between groups.

Conclusions
A single infusion of ZA has favourable effects on the progression of early PDB in *SQSTM1* mutation carriers.

MeSh on Demand Keywords
Humans, Zoledronic Acid, Diphosphonates, Sequestosome-1 Protein, Quality of Life, Infusions, Intravenous, Depression, Follow-Up Studies, Osteitis Deformans, Mutation, Surveys and Questionnaires, Osteoarthritis, Anxiety Radioisotopes

Primary conflicts of interest

Abbreviations

Abbreviation	Definition
AE	Adverse Events
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANCOVA	Analysis of Covariance
ARC	Arthritis Research Council
AST	Aspartate Aminotransferase
BPI	Brief Pain Inventory
BSALP	Bone-specific alkaline phosphatase
CI	Confidence interval
CTX	C-terminal telopeptide of type I collagen
GGT	Gamma Glutamyl Transferase
GFR	Glomerular Filtration Rate
HADS	Hospital Anxiety and Depression Questionnaire
HRQoL	Health-Related Quality of Life
ITT	Intention-To-Treat
MRC	Medical Research Council
PDB	Paget's Disease of Bone
PRSE	PDB-related skeletal events
PINP	N-terminal propeptide of type I procollagen
SAE	Serious Adverse Events
SD	Standard deviation
SF-36	Short Form (36) Health
ZA	Zoledronic Acid

Synopsis of Study

The normal process of renewal and repair of the skeleton is abnormal In Paget's disease of bone (PDB) causing affected bones to enlarge and weaken, resulting in pain, deformity, bone fractures and deafness. People with PDB often present when the disease is at an advanced stage with irreversible skeletal damage. Early diagnosis and prophylactic therapy could help to improve outcome in the disease. Mutations in a gene called *SQSTM1* predispose to inheritance of PDB in families. People with a family history of PDB were offered genetic testing for *SQSTM1* gene mutations. Of the 350 individuals identified, 222 were enrolled and randomly allocated to receive a single infusion of the bisphosphonate zoledronic acid (ZA) or placebo. Both groups were followed up for an average of 84 months.

At baseline, 21/222 individuals (9.5%) already had evidence of PDB on bone scans. Two individuals allocated to placebo developed new bone lesions compared with none allocated to ZA. Eight participants in the placebo group had a poor outcome (lesions which were new, unchanged, or progressing) compared with none in the ZA group (OR =0.08, 95% CI 0.00-0.42, 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 emergence of symptoms related to PDB. There was no significant difference between the groups in quality of life, bodily pain, or anxiety and depression.

The trial has shown that genetic testing coupled with ZA treatment can favourably modify the evolution of PDB in those with *SQSTM1* mutations. The offer of genetic testing for *SQSTM1* mutations coupled with bone scans and targeted intervention with ZA could be of clinical benefit in those individuals with a family history of PDB.

2. Introduction

2a. 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 approximately 1% of British people over the age of 55. It is characterised by focal increases in osteoclastic bone resorption, coupled to increased and disorganised bone formation at one or more sites throughout the skeleton. Although some patients are asymptomatic many others develop complications such as bone deformity, pathological fracture, deafness and secondary osteoarthritis (1). These complications can cause loss of mobility and independence, and adversely affect quality of life (2, 3).

Genetic factors are important in PDB, and the disease can be inherited as an autosomal dominant trait in some families (4-6). Mutations have been identified in four genes that predispose to PDB and related conditions (7), but the most important of these is *SQSTM1* which encodes p62; a scaffold protein in the NF B signalling pathway (8-10). Between 20-50% of patients with a family history of PDB carry *SQSTM1* mutations and the mutations also occur in between 5-20% of patients without a known family history of the disease (11-17). Individuals with mutations of the SQSTM1 gene are at high risk of developing PDB which has an early age of diagnosis and is more clinically severe than those without the mutations (18). Penetrance is about 90% 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 sometimes 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 (24, 25) which can result in a sustained biochemical remission of the disease in over 95% of subjects for up to 6.5 years following a single injection (26).

2b. Objectives

The **primary** objective of the ZIPP trial was to determine if targeted intervention with Zoledronic acid 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 determine whether targeted intervention with Zoledronic acid can:

- Modify the activity of existing bone lesions in carriers of *SQSTM1* gene mutations
- Reduce or prevent PDB-related skeletal events (PRSE) in carriers of *SQSTM1* mutations.
- Reduce or prevent increases in bone turnover in carriers of *SQSTM1* gene mutations.
- Modify quality of life, bone pain and anxiety or depression

3. Methods

3a. Trial Design

This study was a multi-centre double blind, placebo controlled, randomised trial of intravenous Zoledronic acid 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 underwent genetic testing for SQSTM1mutations 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, first-degree relatives of these individuals (primarily children) were asked to undergo genetic testing for the study. Individuals who consented to undergo testing and were found to be positive for SQSTM1 mutations were then 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 Zoledronic acid 5mg or an identical placebo by intravenous infusion. Participants who tested negative for *SQSTM1* mutations were invited to take part in the observational study were invited to take part in an 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 imaged by radionuclide bone scans to look for any evidence of PDB. They were contacted by telephone one 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 and anxiety and depression by questionnaires. Blood samples were taken for routine biochemistry and biochemical markers of bone turnover. At the end of study visit, the 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 on the next page of the report.

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*	✓	✓		✓	✓
Routine Haematology†		✓			✓
Blood for specialised biomarkers‡		✓		✓	✓
Urine for specialised biomarkers§		✓			✓
SQSTM1 genotyping	✓				
25(OH) vitamin D	✓				
Pregnancy test		✓			
Radionuclide bone scan		✓			✓
Radiographs or other imaging**		✓			✓
Infusion		✓			
Telephone review			✓		
Food Frequency		✓			
SF-36, HADS & BPI PDB-related skeletal events		✓		✓	✓ ✓

** Calcium, albumin/total protein, alkaline phosphatase, liver function (AST, ALT, GGT, bilirubin), urea and electrolytes and creatinine. †Full blood count. ‡Blood samples for measurement of bone-specific alkaline phosphatase, PINP, CTX-I and other specialised markers of bone metabolism. §Second-voided morning urine was taken and stored for measurement of N-telopeptide collagen cross links, deoxypyridinoline/creatinine ratio and other specialised markers of bone metabolism. ¶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. **To be taken of relevant areas in subjects suspected to have PDB-like bone lesions on bone scan. ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BPI, Brief Pain Inventory; CTX-I, C-terminal telopeptide of type I collagen; GGT, Gamma glutamyl transferase; HADS, Hospital Anxiety and Depression Questionnaire; hCG, Human chorionic gonadotrophin; PDB, Paget's disease of bone; PINP, N-terminal propeptide of type I procollagen; SF-36, Short Form (36) Health Survey ; SQSTM1, sequestosome-1; ZiPP, Zoledronic acid in the Prevention of Paget's disease.*

Radionuclide bone scan

Bone lesions were assessed by Tc^{99} radionuclide bone scan, which is recognised to be the most sensitive imaging technique for identifying bone lesions in PDB (27, 28). Participants thought to have PDB-like bone lesions on scan had further imaging performed by X-ray, CT scan or 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 disagree on a specific image, a third imaging expert (also blinded to treatment allocation) was asked to adjudicate.

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 was performed using standard techniques at the local laboratories in participating centres. The estimated glomerular filtration rate (GFR) was calculated from serum creatinine, gender and weight by the Cockcroft-Gault equation (29).

Specialised Biochemical Markers

Specialised biochemical markers of bone turnover were measured centrally at the University of East Anglia. These included urine N-telopeptide collagen cross links (NTX) corrected for urinary creatinine; C-terminal telopeptide of type I collagen (CTX-I), bone-specific alkaline phosphatase (BSALP) 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 (30). The urine samples were second-voided ‘spot’ samples collected after an overnight fast. The preferred markers of bone resorption are urinary NTX and serum CTX-I. These have been found to be elevated in patients with PDB in case–control studies (31) and to correlate with the extent of bone lesions as determined by scintigraphy in PDB (27, 32) The markers of bone formation used were PINP and BSALP since both have been shown to be superior to total ALP at detecting PDB in case–control studies (27).

Health-related quality of life

At all indicated visits, the participants Health-related quality of life was assessed by the completion of the Short Form (36) Health Survey (SF-36) questionnaire. The SF-36 is a widely used, validated questionnaire (33) previously used to assess quality of life in patients with established PDB (34, 35)

Brief Pain Inventory

The presence and location of pain was assessed by completion of the Brief Pain Inventory (BPI) (36) . 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 (37). In addition to completing BPI, participants were also asked if they had experienced any pain and bone pain

Hospital Anxiety and Depression Questionnaire

Anxiety and depression was assessed by the Hospital Anxiety and Depression Questionnaire (HADS) (38). This questionnaire was chosen since it was quick and simple to administer and it has been extensively validated in many different countries and settings (39).

Paget's disease-related skeletal events (PRSE)

Participants were evaluated clinically at the end of study for the presence of Paget's disease-related skeletal events (PRSE). 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 as will the development of new bone lesions on bone scan. All events will be combined for each treatment group to give a total score.

3b. Changes to trial design

In the original protocol, participants in the active treatment arm were to have a second infusion of the study IMP at 30 months to further suppress bone turnover. However, studies by Reid et al 2011 (25), have shown that a single infusion of Zoledronic acid can suppress bone turnover in PDB for at least 6.5 years. Therefore, indicating 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 Zoledronic acid SmPC. This involved removing the abnormalities of liver function as exclusion criteria since Zoledronic acid is not contraindicated in patients with liver disease and can be used without adjustment in patients with abnormal liver function. The exclusion criteria of hypocalcaemia was amended, it was originally an exclusion criterion with a cut off value of <2.2mmol/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 <2.2mmol/l was removed but hypocalcaemia, as defined by the local laboratory reference range, was retained as an exclusion criterion because hypocalcaemia is a contra-indication to the use of Zoledronic acid. The trial was extended by 22 months to 31st 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.

4a. Participants

Probands were eligible for genetic testing if they had been diagnosed with PDB and had any relatives that were aged 30 years or older, 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.

4b. Study setting

This was a multi-centre trial that was conducted at 27 secondary care referral centres for bone disease in 7 countries. All the visits were conducted within a secondary health care setting. Table 2 summarise the sites that enrolled participants into the ZiPP trial.

Table 2: Summary of site locations that enrolled participants into the ZiPP study.

Country	City
United Kingdom	Edinburgh
	London-Guys
	Manchester
	Oswestry
	Liverpool
	Bristol
	London - King's
	Portsmouth
	Nottingham
Ireland	Dublin
Spain	Barcelona - Hospital Clinic
	Barcelona - Hospital del Mar
	Salamanca
Italy	Turin
	Siena

	Florence
Belgium	Brussels
Australia	Perth
	Geelong
	Newcastle
	Toowoomba - St Vincent's Hospital,
	Sydney
	Brisbane
New Zealand	Auckland
	Christchurch

5. Interventions

The IMP or placebo was given by a single intravenous infusion and comprised of either zoledronic acid (Aclasta®) (5 mg in 100 mL ready-to-infuse solution) or a matching placebo (0.9% saline). Both were given at a constant infusion rate over not less than 15 min.

6a. Outcomes

Primary outcome measures

The primary outcome measure was the total number of subjects who develop new bone lesions on radionuclide bone scans with the characteristics of PDB between the baseline visit and the final follow up visit. The presence of such lesions would be assessed by an imaging expert blinded to treatment allocation. 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 which 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 assessed. 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 which 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 based on the method described by Patel et al. (40)
3. The development of PDB-related skeletal events (PRSE) 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, 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 (uNTX/Cr and CTX) and bone formation (ALP, BSALP, P1NP). These markers were measured centrally at the University of East Anglia 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 (33), BPI (37) and HADS questionnaires (39). 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

6b. 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 endpoint as described in subsection 3 of the previous page.

7a. 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 will 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 90% of patients with established PDB (41) 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 alkaline phosphatase values in patients within families who carried the same mutation (271.3) and the mean squared alkaline phosphatase 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.

7b. Interim analyses and stopping guidelines
Not applicable

8a. 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, including which study the participant was to be randomised into. Once the participant was enrolled and randomisation had occurred a treatment code was provided. All treatment codes were generated by the drug manufacturer and were built in blocks of 4. This treatment code was then presented to the pharmacy, and treatment provided.

8b. Randomisation: type
Patients were randomised to either Zoledronic acid or matched placebo infusion, with a treatment allocation ratio of 1:1. The randomisation was minimised according to the type of mutation (missense versus 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 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.

9. Randomisation: allocation concealment mechanism
Allocation concealment was assured by the fact that the Zoledronic acid 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 pharmacist.

10. 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

study the participant was to be randomised into. 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 4. This treatment code was then presented to the pharmacy, and treatment was provided.

11a. 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 pharmacy, which had the restricted code break details.

11b. Similarity of interventions
The interventions were 100ml bottles containing clear liquid with an identical appearance. Both were given by intravenous infusion at a constant infusion rate over not less than 15 min.

12a. Statistical methods
The principal analysis was based on the intention-to-treat (ITT) principle incorporating all randomised participants, regardless of treatment received. A binary logistic regression analysis was fitted to compare the number of patients developing new bone lesions between treatment groups. The model included terms for treatment group (Zoledronic acid *vs.* placebo) and it was planned that the model would adjust 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 which resulted in model non-convergence, it was not possible to adjust for the minimisation variables. The number of outcome events was so small that a maximum-likelihood-based logistic regression, either with or without covariate adjustment, was not possible. Therefore Fisher's Exact test was performed, with no covariate adjustment. The effect of randomised treatment was measured by the unadjusted odds ratio (and 95% confidence interval) for Zoledronate vs. placebo.

Secondary outcomes

The secondary outcome measures were:

i. 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 was 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) for Zoledronate vs. placebo.

ii. Change in activity of existing bone lesions that were present at baseline

Change in bone lesion activity was be 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.

iii. Specialised markers of bone turnover

Results of each biomarker sample were modelled using a repeated measures analysis of covariance (ANCOVA) adjusting for the relevant baseline measure and the minimisation variables. The estimated treatment effect and 95% confidence interval were presented for each outcome.

iv. Quality of life questionnaires

The following quality of life measures were formally analysed:

- Brief Pain Inventory (BPI)
- The Short Form Health Survey (SF-36) physical component score mental component score
- Hospital Anxiety and Depression Scale (HADS) interference score, severity score, anxiety score, depression score, total score

A repeated measures analysis of covariance (ANCOVA) adjusting for the relevant baseline QoL measure and the minimisation variables was undertaken. The estimated treatment effect and 95% confidence interval was presented for each outcome.

v. 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. Adverse events were also summarised by bodily system category (musculoskeletal, respiratory, cardiovascular etc.) [No formal testing, Safety population]

Serious adverse events (SAEs) were summarised and listed in line with adverse events. [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 alkaline phosphatase (ALP), a formal analysis of the results was undertaken, using a repeated measures analysis of covariance (ANCOVA) approach. The model adjusted for baseline ALP and the minimisation variables. The estimated treatment effect and 95% confidence interval were presented. [ITT population]

Details were provided of any patients who become pregnant or who have a partner who became pregnant during the study. [No formal testing, Safety population]

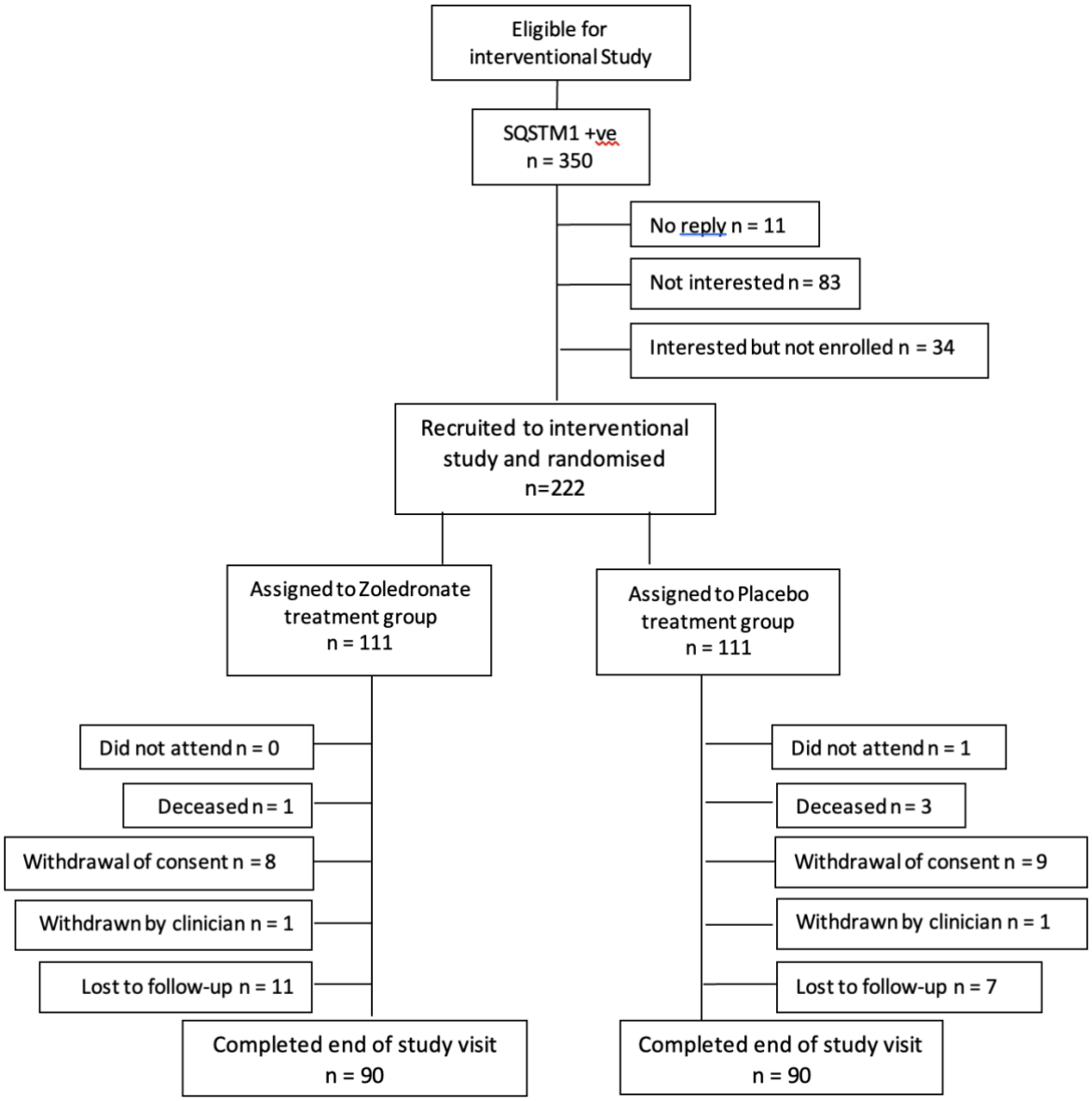
12b. Additional analyses

Not applicable.

13. Results

13a. Participant Flow (consort) diagram

Figure 1: Disposition of participants



13b. Losses and exclusions

The number of participants that 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 Table 3.

Table 3 Summary of withdrawals and deaths

Reason	Zoledronic Acid (N=111)	Placebo (n=111)
Withdrawal of consent - No further contact	5 (4.5%)	7 (6.3%)
Withdrawal of consent - No further access	3 (2.7%)	2 (1.8%)
Withdrawn by clinician	1 (0.9%)	1 (0.9%)
Lost to follow up	11 (9.9%)	7 (6.3%)
Deceased	1 (0.9%)	4 (3.6%)

14a. Recruitment

The first patient was randomised to the study on the 5th March 2010, and the final patient was randomised on the 16th April 2015. There was a total number of 222 participants randomised with 50% (N=111) being allocated to both treatments (Placebo, and Zoledronate 5mg). The recruitment of participants occurred at 25 sites across 7 countries, the distribution of recruitment at each site is shown in table 4.

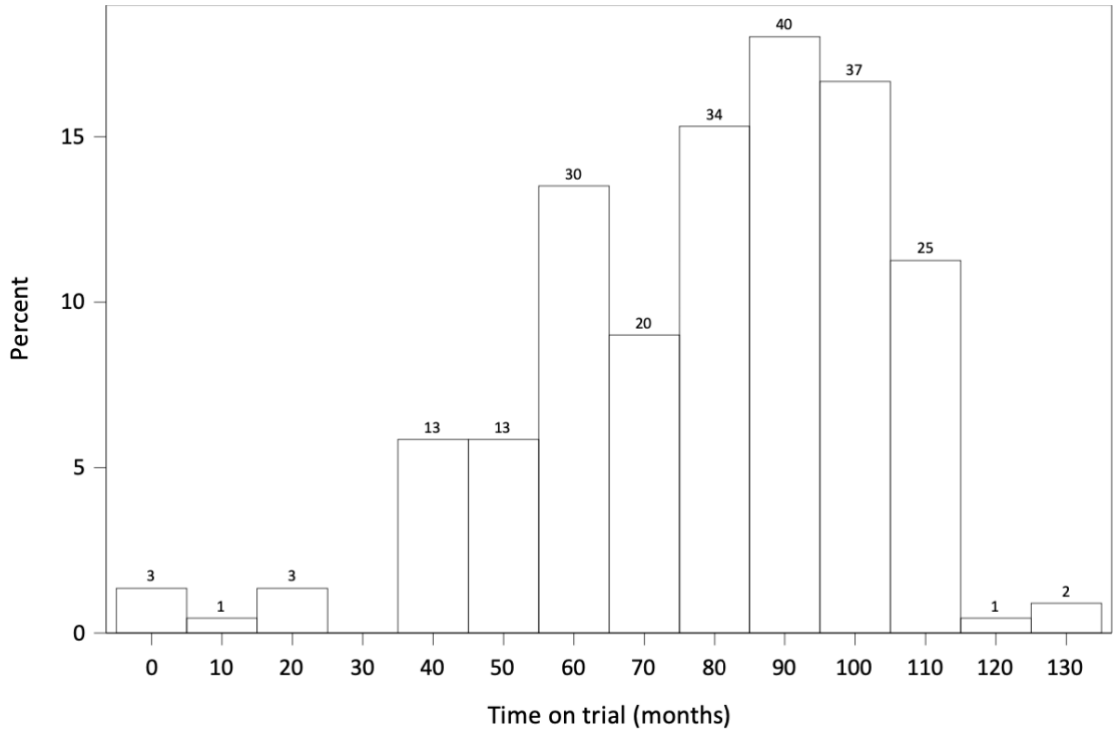
Table 4. Recruitment by site

Location	Zoledronate 5mg (N=111)	Placebo (N=111)
Edinburgh	16 (14.4%)	22 (19.8%)
London-Guy's	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	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%)
Newcastle	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%)

Recruitment to trial ran from 5th March 2010 to 31st December 2021, when the last participant completed their last 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 (SD 24.5) and the Placebo arm 79.0 (SD 24.3). A graphical summary of the duration of follow up in the trial is shown in Fig 2.

Figure 2. Duration of participation in the ZiPP study.



15. 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 5.

Table 5. 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), Mean (SD)	49.8 (8.8)	50.5 (9.3)
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, Mean (SD)		
Weight (Kg)	79.5 (17.7)	82.0 (19.6)
Height (Cm)	168 (9.0)	169 (9.0)
Body Mass Index (BMI)	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/ENT/Eyes		
Normal	106 (95.5%)	108 (97.3%)
Cardiovascular		
Normal	103 (92.8%)	105 (94.6%)
Musculoskeletal		
Normal	101 (91.8%)	101 (91.0%)
CNS		
Normal	109 (98.2%)	108 (97.3%)
Numbers are N (%), unless otherwise stated.		

The results of routine biochemistry and haematology at baseline are shown in Table 6. Mean values were similar in both treatment groups except for Gamma GT which was slightly higher in the Placebo treatment arm (37.9, SD 50.6)) compared with the ZA arm (27.7, SD 17.3)

Table 6. Routine biochemistry and haematology in the study population

	Zoledronate (n=111)	Placebo (n=111)
Raised ALP, N (%)	4 (3.6%)	4 (3.6%)
Alkaline Phosphatase (U/L)	78.2 (41.7)	80.1 (53.1)
Alkaline Phosphatase (adjusted) [1]	0.44 (0.32)	0.47 (0.37)
Calcium (adjusted) (mmol/L) [2]	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)
Gamma GT (U/L)	27.7 (17.3)	37.9 (50.6)
Bilirubin (µmol/L)	10.23 (5.66)	10.40 (5.86)
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)	5.22 (1.35)	5.17 (1.55)
eGFR	86.1 (21.1)	83.3 (17.4)
Routine Haematology		
WBC (10^9/l)	6.36 (1.55)	6.21 (1.69)
Haemoglobin (g/L)	153 (136)	174 (194)
Platelets (10^9/l)	243 (57)	240 (63)
Numbers are Mean (SD), unless otherwise stated.		

ALP = total alkaline phosphatase; AST = Aspartate aminotransferase; ALT = alanine transaminase [1] Adjusted results are expressed in relation to the upper limit of normal for the local reference range. [2] Adjusted for albumin values.

The percentages of participants in each treatment arm that were either above or below the limit for each biochemistry measure is shown in Table 7.

Table 7. Assessment of biochemical reference ranges

	Zoledronate 5mg (N=111)	Placebo N=111
Vitamin D3 (nmol/L)		
Deficient (<25)	10 (9.0%)	10 (9.0%)
Sufficient (25-50)	39 (35.1%)	30 (27.0%)
Normal (>50)	61 (55.0%)	71 (64.0%)
uNTX/Cr (limit = 65)		
Above limit	30 (27.0%)	39 (35.1%)
Below limit	73 (65.8%)	61 (55.0%)
CTX (ng/mL) (limit = 0.704 m/1.018 w)		
Above limit	2 (1.8%)	1 (0.9%)
Below limit	101 (91.0%)	100 (90.1%)
BSALP (U/L) (limit = 42) - Baseline Visit		
Above limit	1 (0.9%)	1 (0.9%)
Below limit	102 (91.9%)	99 (89.2%)
P1NP (ng/mL) (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.		

Details of mutations in SQSTM1 are shown in Table 8. 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).

Table 8. 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 [1]	5 (4.5%)	3 (2.7%)
p.Glu396Ter	0 (0.0%)	1 (0.9%)
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%)	2 (1.8%)
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%)
c.1165+1G>A*	5 (4.5%)	3 (2.7%)
p.Glu396Ter	0 (0.0%)	1 (0.9%)

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

Previous fracture history was assessed at baseline as summarised in Table 9. 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 bone regions (n=31, 14.0%) not categorised in the list.

Table 9. 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 percent

16. 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 were 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, and 7 (6.3%) lost to follow up; and 1 (0.9%) who 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.

17a. 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 10.

Table 10. 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%)

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. (p= 0.246, odds ratio: 0.406, 95% CI 0.000, 3.425). The odds ratio of less than 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.

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 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 8 in the Placebo group (OR =0.08, 95% CI 0.00-0.42, p=0.003). A summary of the changes in bone lesions which occurred during the trial is presented in Table 11.

Table 11. 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 (41.3%)
No change	0 (0%)	8 (27.5%)
Increased	0 (0%)	4 (13.8%)
No end of study assessment	0 (0%)	4 (13.8%)

A summary of change in bone lesions at the individual patient level is summarised in Table 12.

Table 12. 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 [1]	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%)

[1] One participant in the placebo group who required rescue therapy with ZA had 4 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 13 and 14 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 4 lesions at baseline, affecting the left pubic ramus, cervical vertebrae 4 and 5, 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%), two (13.3%) had diminished in activity and no new lesions developed.

Table 13. 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 14. 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**

PDB-related skeletal events (PRSE)

The PDB-related skeletal events (PRSE) reported by the local PI are shown in (Table 15). This identified 2 PRSE's in the ZA treatment arm compared to 13 in the Placebo treatment arm.

Table 15. Summary of PDB-related skeletal events (PRSE)

	Zoledronate 5mg (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.		

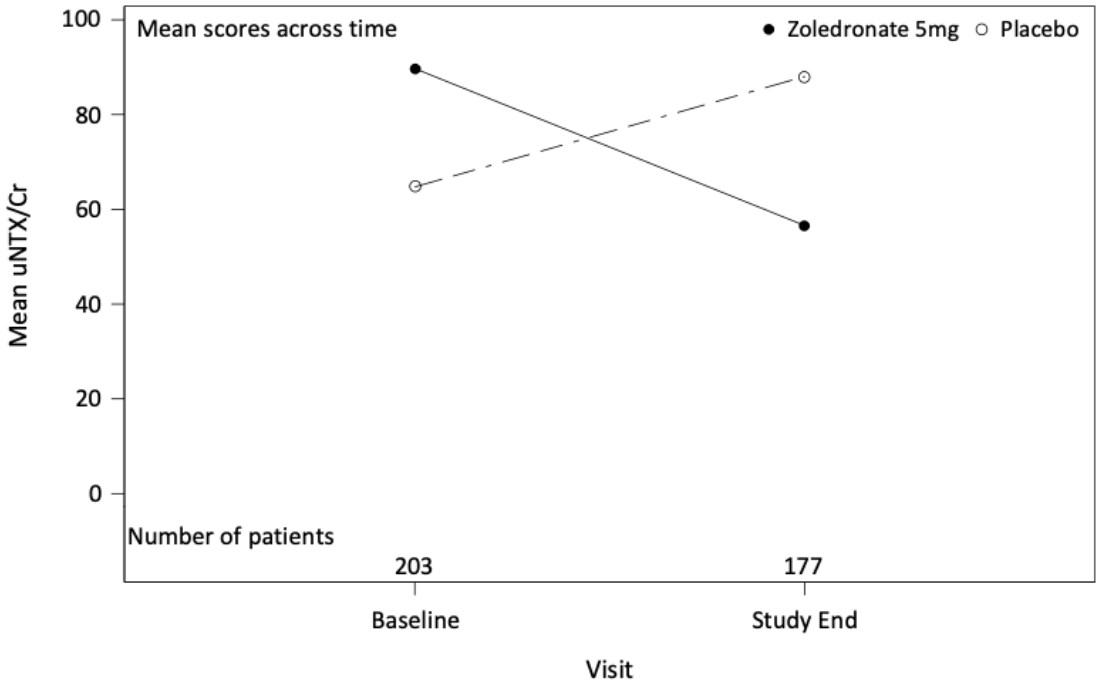
On review of these responses at individual participant level it was noted that most participants did not have PDB lesions on scan at either the beginning or end of study suggesting that there had been a misunderstanding of the definition of PRSE at site level. Because of this two independent adjudicators were appointed to review the PRSE's. Following the adjudication it was concluded that only one participant in the Placebo treatment arm had a PRSE. The PRSE was as 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

Urinary N-telopeptide (uNTX) as a ratio of creatinine - uNTX/Cr

This analyte is a biochemical marker of bone resorption. 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 group, 88.0 (SD 174.8).

Figure 3. Changes in uNTX during the study



Values are means in units of a ratio of Urinary N-telopeptide (uNTX) to creatinine - uNTX/Cr

Serum C-terminal telopeptide - CTX (ng/mL)

Serum CTX is a marker for 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) vs 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 Figures 4a and 4b. (-0.09, 95% CI -0.12,-0.07, P-value <.0001).

Figure 4a. Model based changes in CTX during the study

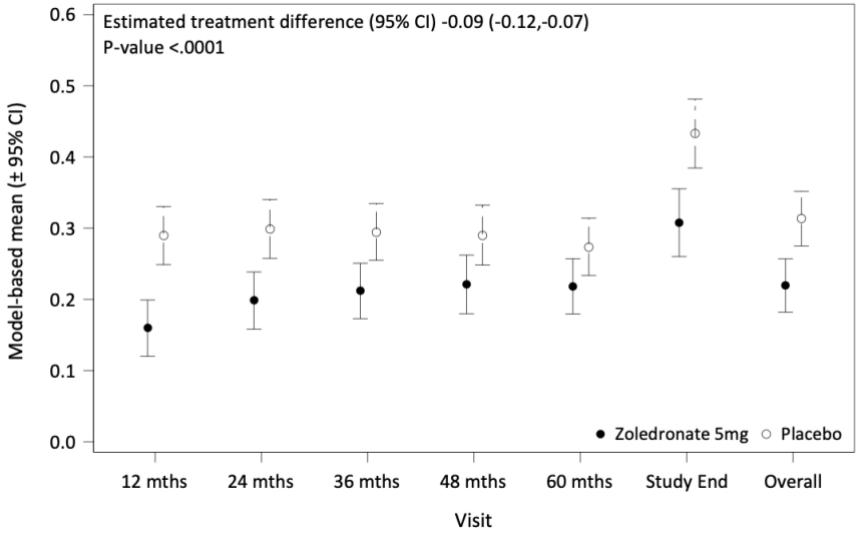
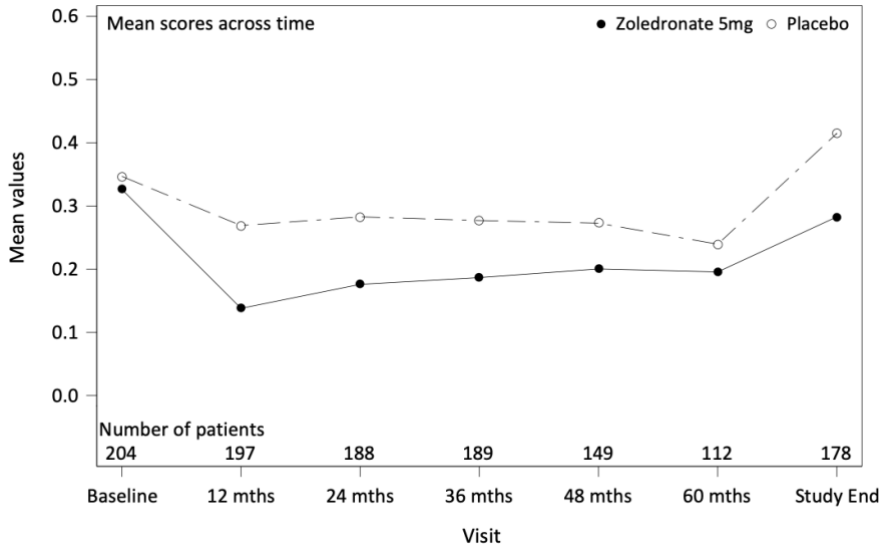


Figure 4b. Mean changes in CTX during the study



Values in ng/mL. Bars in 4a are 95% CI. The number of participants at each time point is shown in Figure 4b

Bone specific alkaline phosphatase (BSALP)

This is a marker of bone formation. Values are shown in Figures 5a and 5b. At baseline, mean values were similar in the two groups, (ZA 11.0 U/L SD 7.5 vs Placebo 10.5 U/L SD 8.0). At the end of study, concentrations of BSALP increased in participants treated with ZA (14.1 U/L SD 5.9), and the Placebo group (17.2 U/L SD 10.2).). Overall, there was a significant reduction in BSALP in the ZA group (-1.68, 95% CI -2.59,-0.78, P-value 0.0003).

Figure 5a. Model based mean changes in BSALP during the study

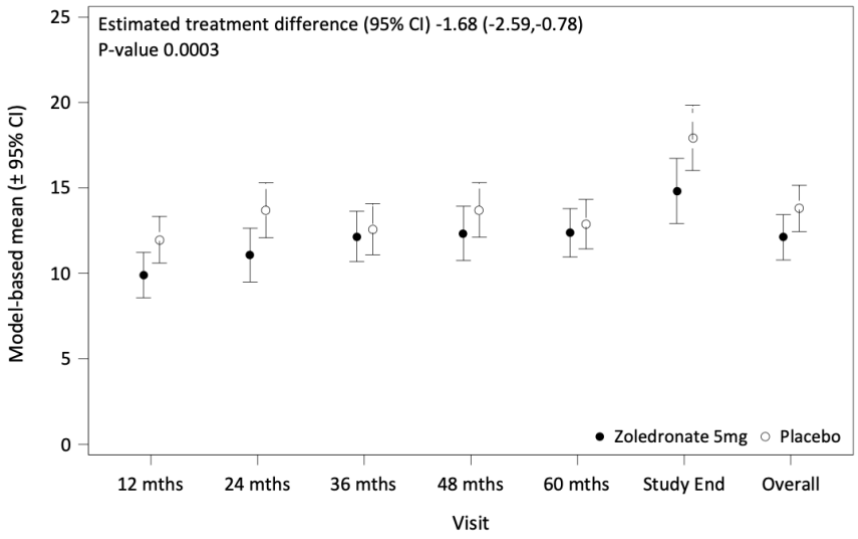
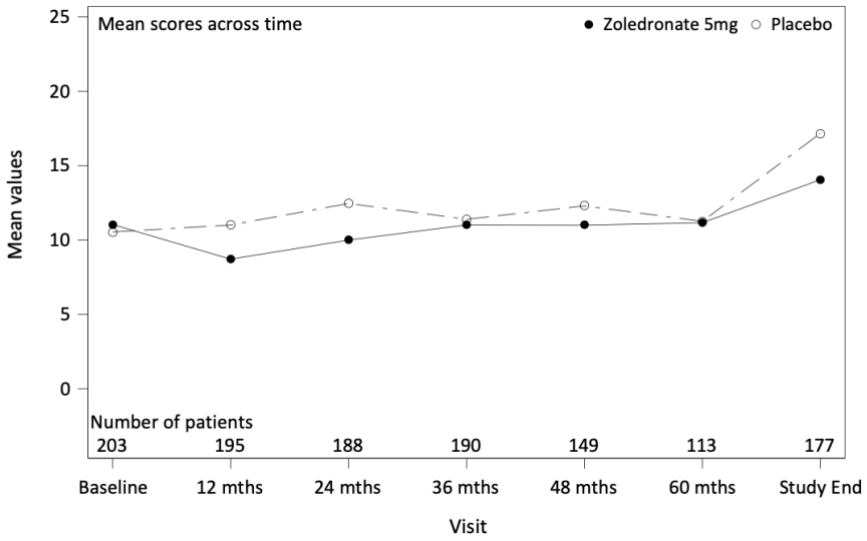


Figure 5b. Mean changes in BSALP during the study



Values are in U/L. Bars in 5a are 95% CI. The number of participants at each time point is shown in Figure 5b

Plasma Procollagen type 1 N-terminal Propeptide (P1NP)

This is a marker of bone formation. Values are shown in Figure 6a and 6b. Mean (SD) baseline P1NP 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, P1NP 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 (-16.32 (-22.05,-10.59), P-value <.0001).

Figure 6a. Model based changes in PINP during the study

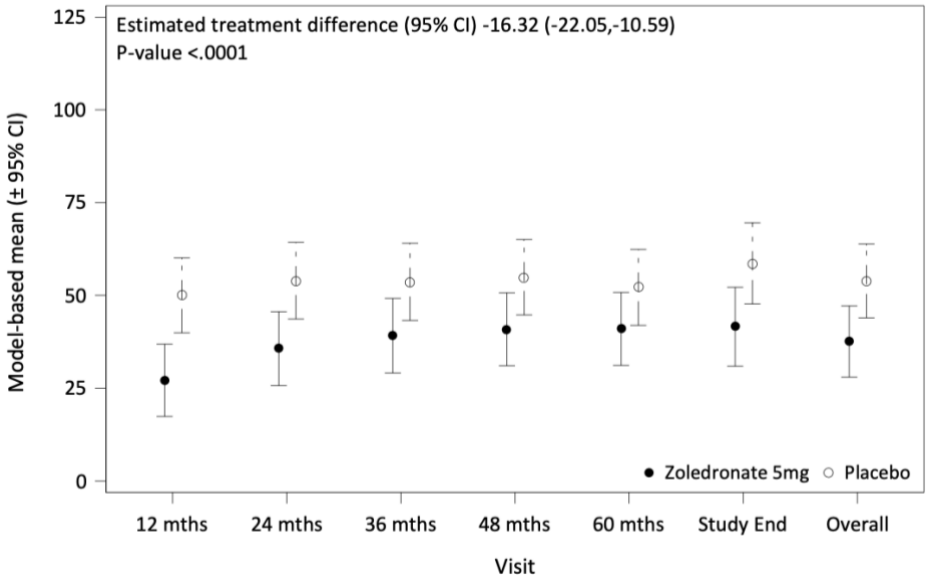
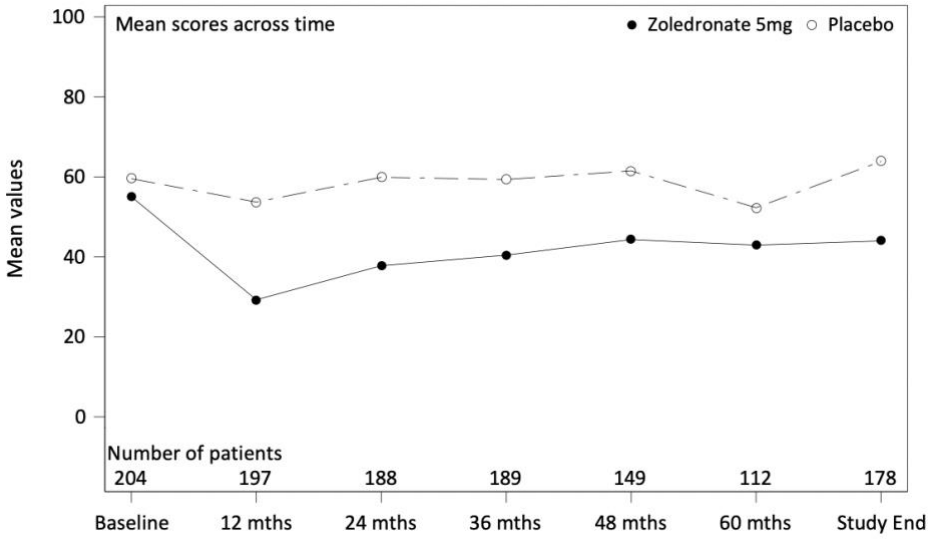


Figure 6b. Mean changes in PINP during the study



Values are ng/mL. Bars in 6a are 95% CI The number of participants at each time point is shown in Figure 5b

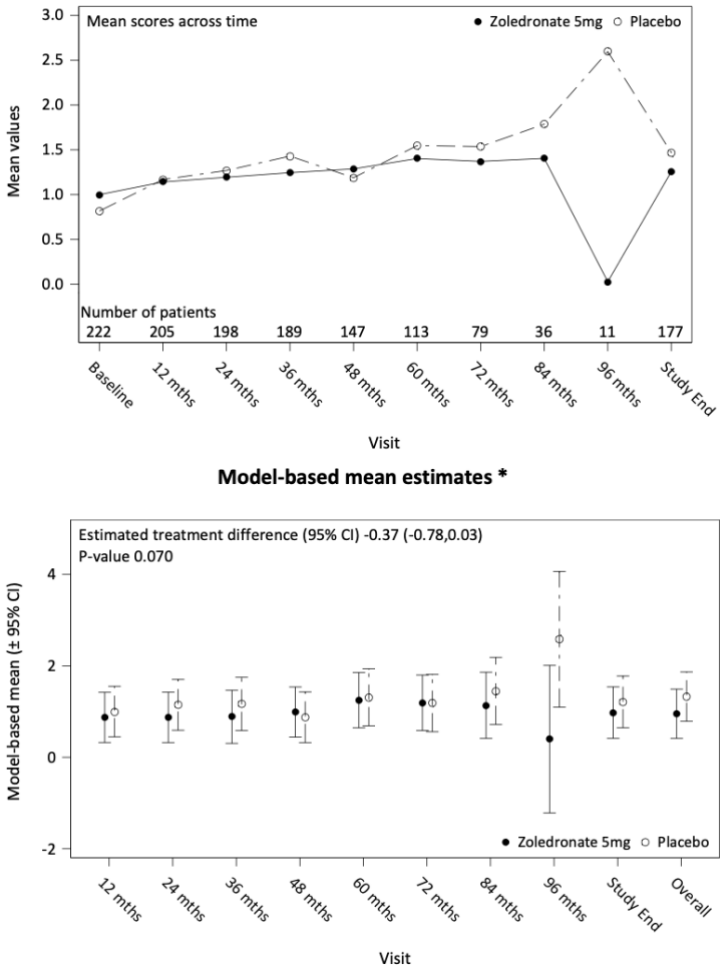
Pain, Quality of Life, Anxiety and Depression
Brief Pain Inventory

Pain was assessed using the Brief Pain Inventory questionnaire. Two components of Pain, interference to life and severity, were measured.

Pain interference score

At baseline the mean interference score was 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 -0.78,0.03, P-value 0.070).

Figure 7. Changes in BPI Interference scores during the study

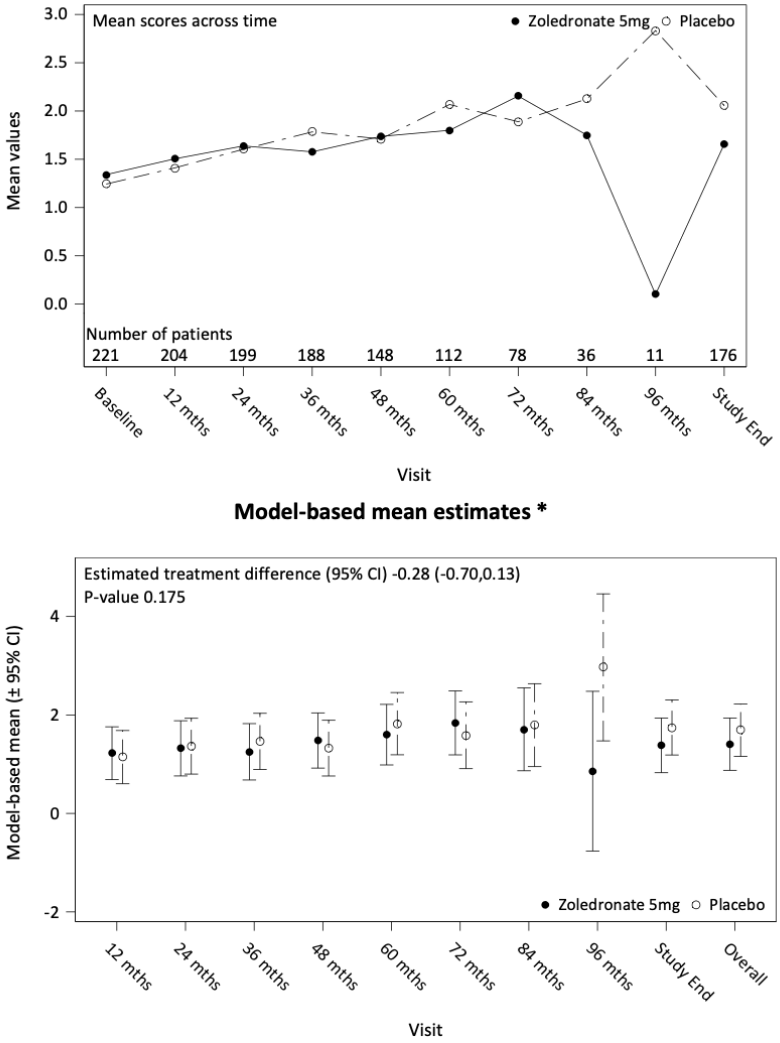


Mean scores with number of observations at each time point are shown in the top panel. Model based estimates with 95% CI are shown in the bottom panel. Scores range between 0 – 10. Higher scores indicate greater pain

Pain severity score

At baseline the mean BPI severity scores were similar in the two groups; ZA 1.34 SD 1.68 vs 1.24 SD 1.53. During the study scores in both groups increased but there was no significant difference between the (-0.28 95% CI -0.70, 0.13, P-value 0.175)

Figure 8. Changes in BPI severity scores during the study



Mean scores with number of observations at each time point are shown in the top panel. Model based estimates with 95% CI are shown in the bottom panel. Scores range between 0 – 10. Higher scores indicate greater pain

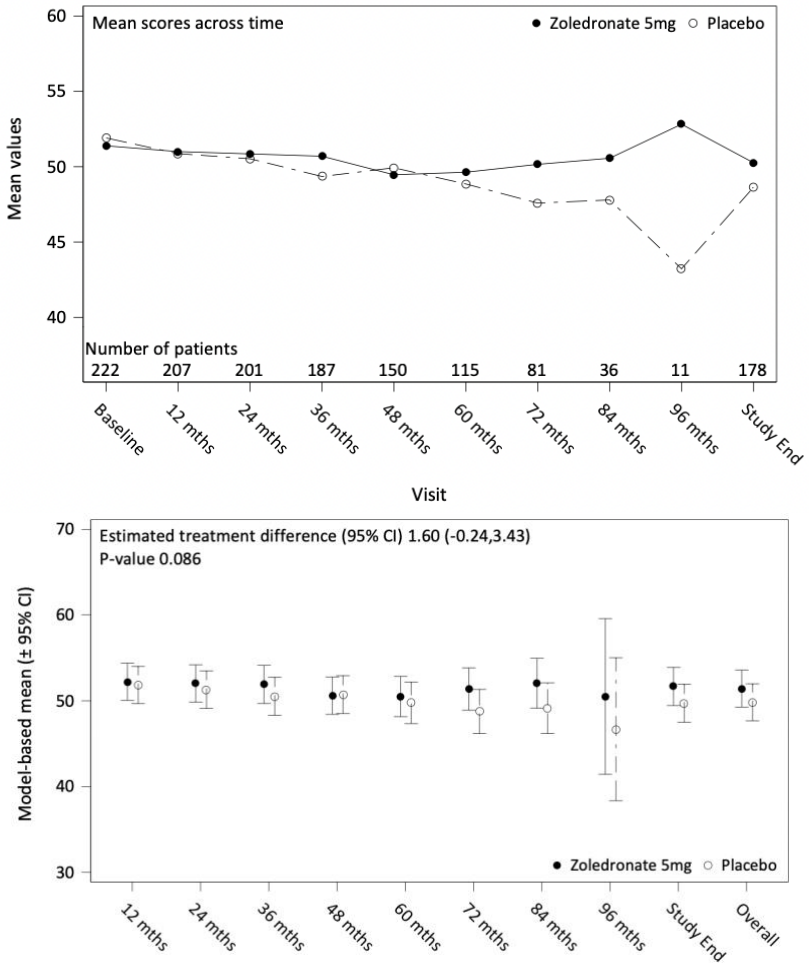
SF36 quality of life questionnaire

The quality of physical and mental components of a participant's life were assessed by the SF36 questionnaire.

Physical component summary

At baseline the mean physical component summary scores were similar in the ZA arm 51.4 (SD 8.1) and placebo arm 51.9 (SD 8.6) (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, 3.43, P-value 0.086).

Figure 9. Changes in SF36 physical component summary during the study

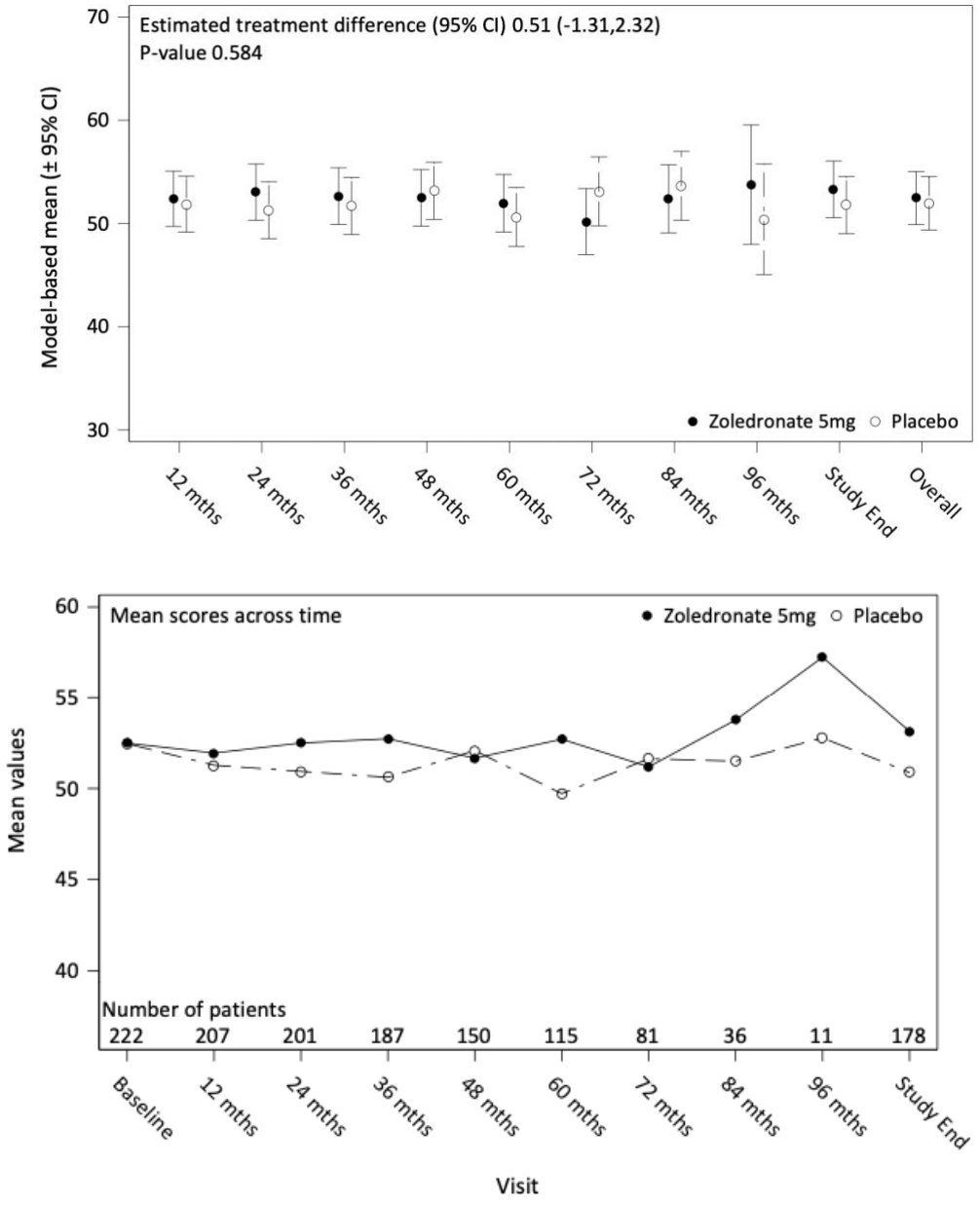


Mean scores with number of observations at each time point are shown in the top panel. Model based estimates with 95% CI are shown in the bottom panel. A score less than 50 indicates health status below average and vice versa.

Mental component summary

Values for the SF-36 - Mental Component Summary Score were identical at baseline with a mean value of 52.5 (SD 8.5) (Figure 10). During the study scores tended to increase in the ZA 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, 2.32, P-value 0.584)

Figure 10. Changes in SF36 mental component summary during the study



Mean scores with number of observations at each time point are shown in the bottom panel. Model based estimates with 95% CI are shown in the top panel. A score less than 50 indicates health status below average and vice versa

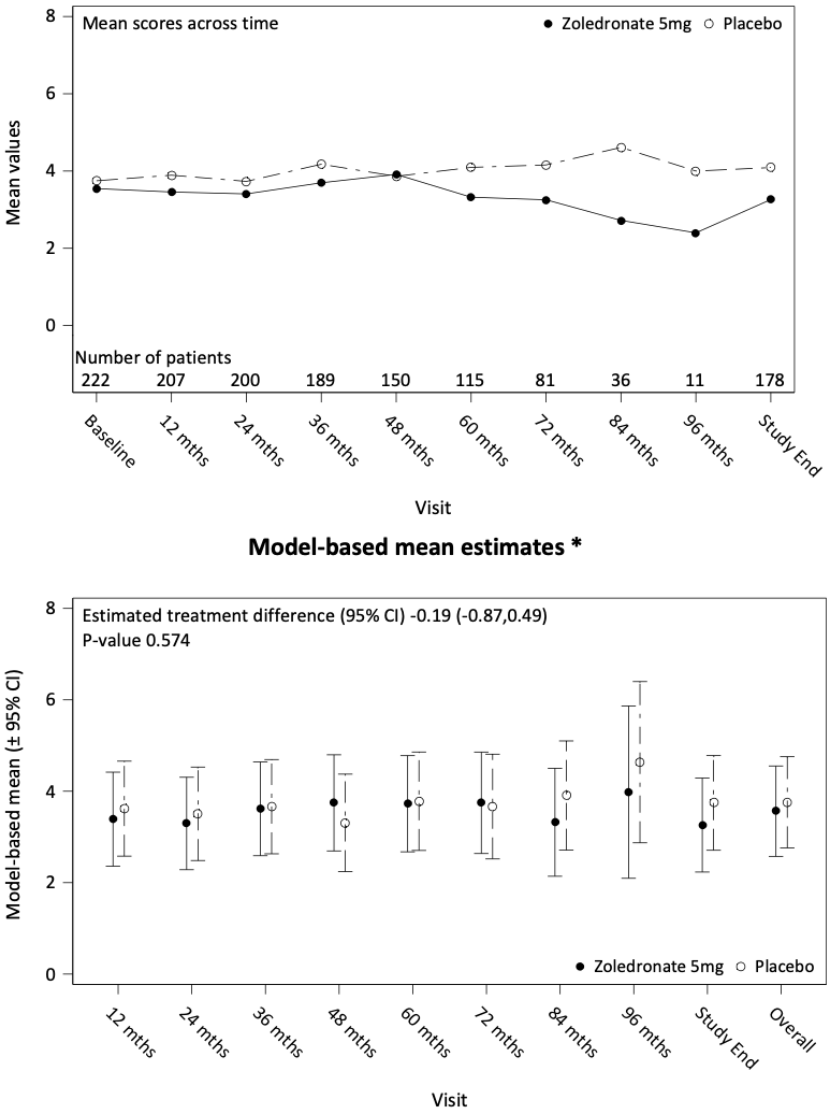
Anxiety and Depression

Anxiety and depression were assessed by the Hospital Anxiety and Depression questionnaire (HADS)

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).

Figure 11. Changes in anxiety scores during the study

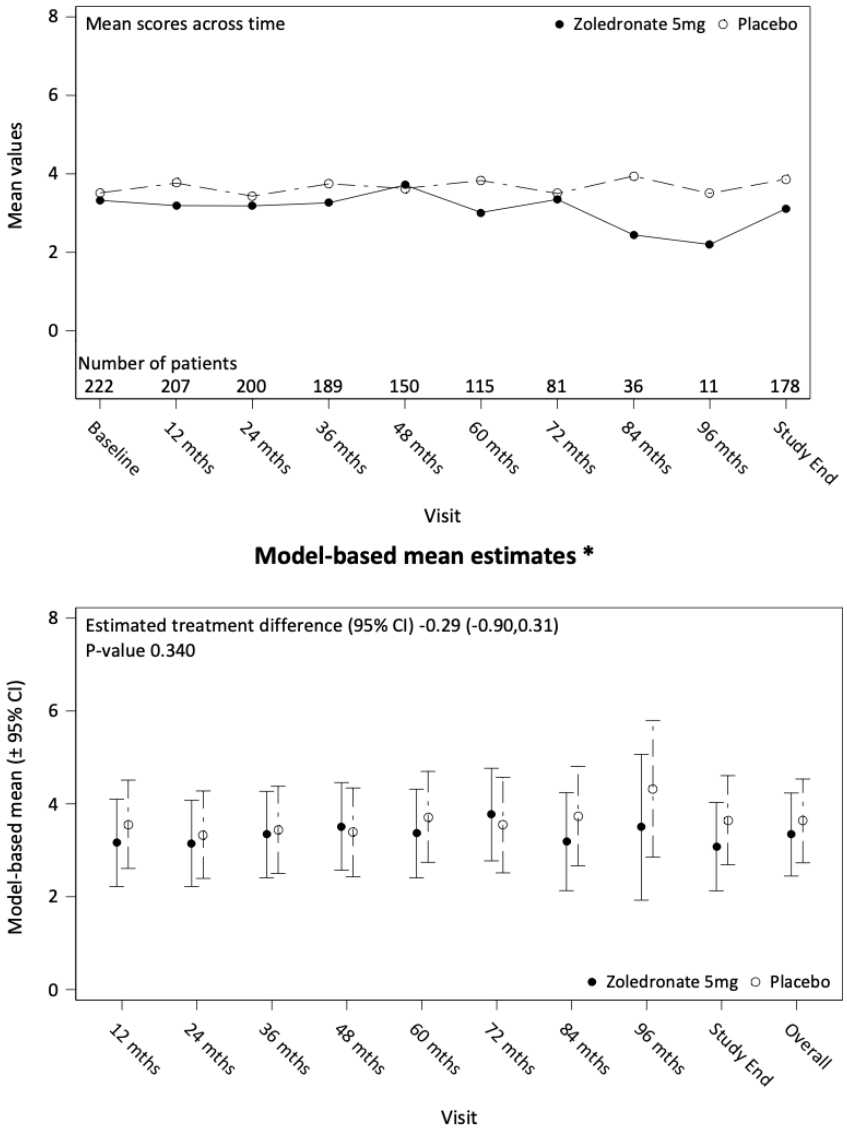


Mean scores with number of observations at each time point are shown in the top panel. Model based estimates with 95% CI are shown in the bottom panel. Higher scores indicate greater anxiety levels.

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, 0.31), P-value 0.340.

Figure 12. Changes in depression scores during the study

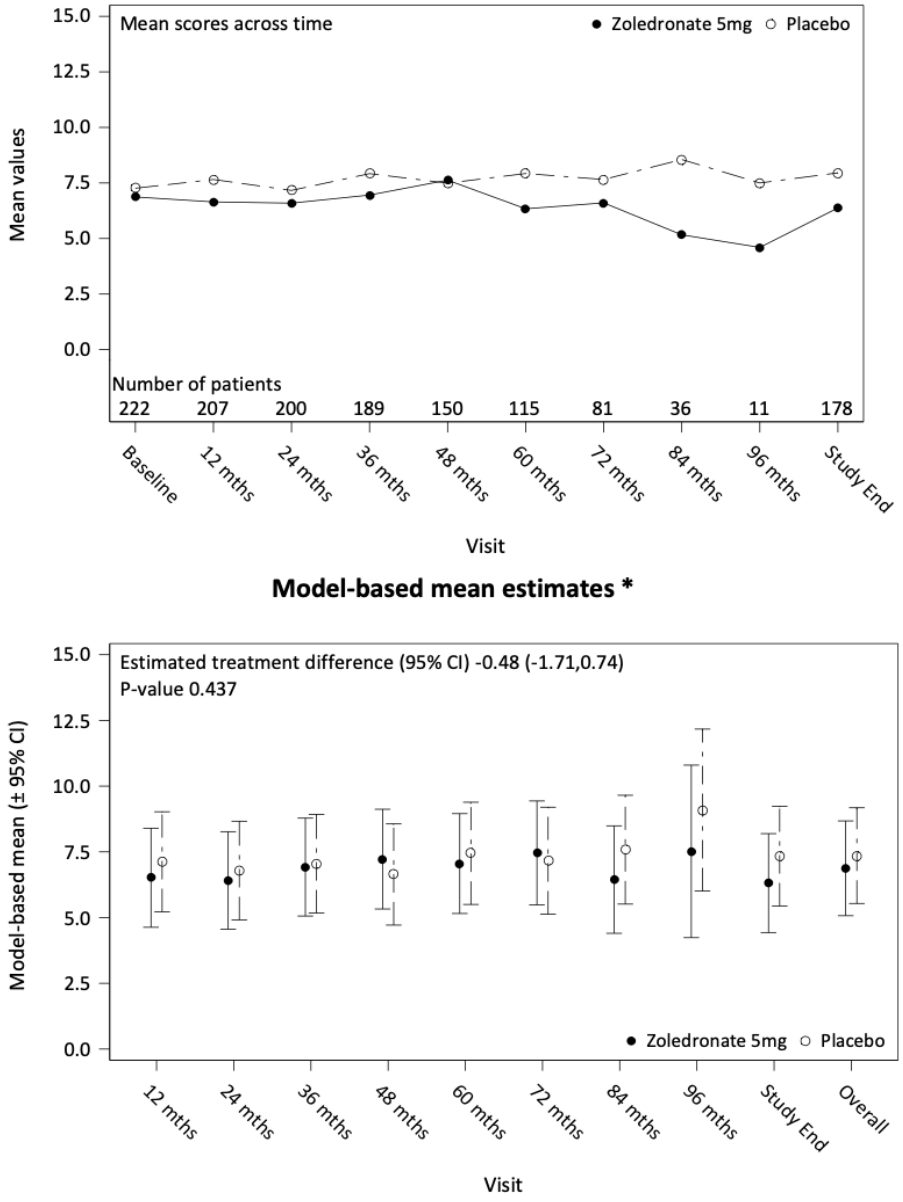


Mean scores with number of observations at each time point are shown in the top panel. Model based estimates with 95% CI are shown in the bottom panel. Higher scores indicate greater anxiety levels.

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 -1.71,0.74) P-value 0.437.

Figure 13. Changes in combined anxiety and depression score during the study



Mean scores with number of observations at each time point are shown in the top panel. Model based estimates with 95% CI are shown in the bottom panel. Higher scores indicate greater anxiety/ depression levels

17b. Binary outcomes
Not applicable

18. Ancillary analyses
Not applicable

19. Harms
The proportion of patients experiencing any adverse event was similar between the ZA and placebo groups: 85 (77.3%) of 111 and 87 (78.4%) of 111, respectively. The number of reported adverse events was similar in patients treated with ZA than in those treated with placebo for 11 of 16 body systems (Table 16). There were 8 (1.4%) adverse events in the ZA treated group that were judged to be directly related to the drug, while in the Placebo treated group there were 2 (0.3%) events reported as directly drug-related.

Table 16. Adverse events grouped by study group

	Zoledronic Acid (n=111)	Placebo (n=111)	Total
Total Adverse Events	583	644	1,227
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%)	7 (0.6%)
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%)
Musculoskeletal 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%)
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%

The adverse events are categorised by the Medical Dictionary for Regulatory Activities – (MedDRA) system organ classes. Values are number and percentages for the events reported.

The proportion of patients experiencing a serious adverse event, as judged by the investigators, was numerically lower in the ZA group than in the Placebo group: 18 (14.3%) of 111 versus 25 (19.1%) of 111 patients, respectively. The number of reported serious adverse events was lower in patients treated with ZA than in those treated with placebo for 12 of 14 MedDRA System Organ Classes (Table 17). There were no serious adverse events reported as being suspected to be drug-related.

Table 17: MedDRA coding of Serious adverse events

MedDRA System Organ Class (**SOC**)	Zoledronate 5mg (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%)
Musculoskeletal 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%)

The serious adverse events are categorised by the Medical Dictionary for Regulatory Activities – (MedDRA) system organ classes. Values are number and percentages for the events reported.

Discussion

20. Limitations

There were two notable limitations to the study. The first was that we did not anticipate that about 10% of participants would have already shown bone scan evidence of Paget's disease at the baseline visit. Since the primary endpoint was the number of participants with new lesions with the characteristics of PDB this reduced 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 2 participants developed new lesions compared with the 15% anticipated assuming a 5-year follow up. It should be noted that this estimate of 15% with emergent bone lesions was based on limited data from cross sectional studies and the known trajectory of increase in the incidence of PDB with age. Nonetheless we were able to show a clear treatment effect on evolution of existing lesions (see below).

21. Generalisability

We believe that the findings are generalisable to individuals with a family history of Paget's disease who are willing to undergo genetic testing for SQSTM1 mutations. We 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.

22. Interpretation

Although the primary endpoint 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 one lesion disappeared (3.4%), 12 were thought to have improved (41.3%); 8 were thought to have remained static (27.5%) and 4 to have progressed (13.7%). Since previous studies in PDB have shown that lesions seldom progress to involve new bones in PDB, we consider a "stable" lesion on bone scan to be a poor outcome.

Another important point to emerge from the study was that the intervention with ZA was very well tolerated with an overall balance of AE and SAE which was almost identical between the study groups. This also held true when we looked at the number of AE which were reported at the telephone review at 1 week post infusion. Our 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 in this study, 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 84

months follow up. So delivery of this intervention would be eminently feasible in routine clinical practice.

We believe that the ZIPP study may provide an impetus to introduce a programme of genetic testing for SQSTM1 mutations coupled with bone scan examination with the offer of intervention with ZA in people who have a family history of PDB.

23. Registration

This study was registered with ISRCTN with a number of ISRCTN11616770

24. Protocol

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

25. Funding

Funding for the study was provided by the Medical Research Council (MRC) (UK) and Arthritis Research Council (ARC) (UK). The IMP, Zoledronic acid (Aclasta®) 5mg, and its subsequent labelling and packaging was supplied by Novartis Pharmaceuticals UK Limited

26. Acknowledgements

26.1 Contributions of authors'

"Professor Stuart Ralston (Versus Arthritis Professor of Rheumatology, University of Edinburgh) was the Chief Investigator of the study, conducted the analysis of the Bone Scintigraphy images, and co-wrote the case report study."

"Professor Steff Lewis (Professor of Medical Statistics, University of Edinburgh) was a co-author on the protocol and Statistical Analysis Plan and developed the trial design and methodology alongside other co-applicants.

"Mrs Catriona Keerie (Senior Research Fellow, University of Edinburgh) conducted the statistical analysis."

"Dr Deepak Subedi (Consultant Radiologist, NHS Lothian) conducted the analysis of the Bone Scintigraphy images."

"Dr Jonathan Phillips (Trial Manager, University of Edinburgh) co-wrote the case report study."

26.2 Publications

Cronin O, Subedi D, Forsyth L, Goodman K, Lewis SC, Keerie C, Walker A, Porteous, M, Cetnarskyj R, Ranganath LR, Selby PL, Hampson G, Chandra R, Ho S, Tobias JH, Young-Min SA, McKenna MJ, Crowley RK, Fraser WD, Tang J, Gennari L, Nuti R, Brandi ML, Del Pino-Montes J, Devogelaer JP, Durnez A, Isaia GC, Di Stefano M, Rubio JB, Guanabens N, Seibel MJ, Walsh J P, Kotowicz MA, Nicholson GC, Duncan EL, Major G, Horne A, Gilchrist NL, Ralston SH. 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. doi: 10.1002/jbmr.4007. Epub 2020 Apr 20. PMID: 32176830.

Cronin O, Forsyth L, Goodman K, Lewis SC, Keerie C, Walker A, Porteous M, Cetnarskyj R, Ranganath LR, Selby PL, Hampson G, Chandra R, Ho S, Tobias JH, Young-Min S, McKenna MJ, Crowley RK, Fraser WD, Gennari L, Nuti R, Brandi ML, Del Pino-Montes J, Devogelaer JP, Durnez A, Isaia G, Di Stefano M, Guanabens N, Blanch J, Seibel MJ, Walsh JP, Kotowicz MA, Nicholson GC, Duncan EL, Major G, Horne A, Gilchrist NL, Boers M, Murray GD, Charnock K, Wilkinson D, Russell RGG, **Ralston SH.** 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.

27. Acknowledging the use of patient data

This work uses data provided by patients and collected by the NHS and secondary health care 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's important that there are safeguards to make sure that it is stored and used responsibly. Everyone should be able to find out about how patient data is used. You can find out more about the background to this citation here: https://understandingpatientdata.org.uk/data-citation

28. Data Sharing

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

29. 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 Research Ethics Committee on 22 December 2008 (08/S0501/84).

30. 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.

31. Equality, Diversity and Inclusion Statement

No restrictions were placed on recruitment to the study based on ethnic background or sex. Recruitment was restricted to individuals above 30 years because Paget's disease increases in incidence with age. It was therefore considered that recruitment of those under the age of 30 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 zoledronic acid.

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