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Effect of denosumab on osteolytic lesion activity after total hip arthroplasty: a single-centre, randomised, double-blind, placebo-controlled, proof-of-concept superiority trial

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ABSTRACT

Background Osteolysis causes recurrent pain and disability after total hip arthroplasty (THA). We investigated the effect of the human monoclonal antibody denosumab on osteolytic lesion activity in patients undergoing revision THA surgery to demonstrate the biological proof-of-concept for a non-surgical treatment for the disease.

Methods We did a phase two, randomised, double-blind, placebo-controlled superiority trial at Sheffield Teaching Hospitals (Sheffield, England). Eligible patients aged 30 years or older and scheduled for revision surgery for symptomatic osteolysis were randomly allocated (1:1) to subcutaneous denosumab (60mg single-dose) or placebo by an independent pharmacist using a random number table. The primary outcome was the between-group difference in osteoclast number/mm of bone surface of biopsies taken from the osteolytic membrane-bone interface at surgery eight weeks later, measured by quantitative histomorphometry. Adverse events were analysed in all randomised participants. This trial is registered with the EU Clinical Trials Register (EudraCT 2011-000541-20).

Findings Between December 19, 2012 and June 24, 2018, 51 patients were reviewed for eligibility, of whom 24 were randomly assigned to study treatment. Two had their revision surgery cancelled for unrelated reasons, leaving 22 participants (ten denosumab) for analysis of the primary outcome. There were 83% fewer osteoclasts at the osteolysis membrane-bone interface (median 0·05/mm [IQR 0·11] versus 0·30/mm [0·40], p=0·011) in the denosumab versus the placebo group. No deaths or treatment-related serious adverse events occurred. In four of 11 participants randomised to denosumab seven adverse events occurred, including one serious adverse event. In five of 13 participants randomised to placebo ten adverse events occurred, including three serious adverse events.

Interpretation To our knowledge, this is the first clinical trial of an investigational drug for osteolysis that demonstrates tissue-specific biological efficacy. These results justify the need for trials that target earlier-stage disease to test for clinical efficacy in reducing the need for revision surgery.

Funding Amgen

RESEARCH IN CONTEXT

Evidence before this study

We searched MEDLINE and PubMed for "total hip arthroplasty" (THA) and "Denosumab", filtering by "clinical trial" and "osteolysis" or "aseptic loosening". We identified no completed trials reporting the use of denosumab to treat periprosthetic osteolysis or aseptic loosening. When the filters "osteolysis" and "aseptic loosening" were removed, we identified 2 recent clinical trials showing the effect of denosumab in maintaining bone mineral density around the prosthesis over the first 2 years after primary surgery, an effect previously established using bisphosphonates. We found no reports on the effect of denosumab on later bone loss, osteolysis, aseptic loosening or revision risk.

Added value of this study

The only established treatment for prosthesis-related osteolysis after joint replacement is revision surgery, which carries substantially greater morbidity and mortality that primary joint replacement. This proof-of-concept study shows that a single 60mg dose of the monoclonal antibody to receptor activator of NFκB ligand, denosumab, is effective in reducing osteoclast number, eroded surface and bone turnover within established, symptomatic osteolytic lesions after THA.

Implications of all the available evidence

These data provide the biological evidence base necessary justify phase three trials in participants with earlier-stage disease to test for clinical efficacy in reducing rates of disease progression and the need for revision surgery. The establishment of such an alternative therapy would reduce the clinical and economic burden caused by joint replacement failure.

INTRODUCTION

Despite ongoing advances in technology resulting in improved survival,[1] prosthesis wear-induced osteolysis leading to loosening remains the most frequent reason for revision surgery to a total hip replacement (THA) across Europe, Australasia, and Canada (Appendix, page 1). For example, in the period January 2014 to December 2018, osteolysis and aseptic loosening accounted for 20,646 of 38,550 (53%) of all revision procedures reported to the NJR in England and Wales.[2] Osteolysis arises as an innate immune inflammatory response to the prosthesis materials and is characterised by the development of a granulomatous membrane at the prosthesis-bone interface.[3] Pro-inflammatory cytokine release from the membrane leads to osteoclast activation, focal bone resorption and prosthesis loosening, resulting in pain and disability that requires revision surgery.[4] Revision surgery is associated with a three-to-eight-fold greater hospital mortality, higher morbidity and risk of re-revision, a smaller improvement in patient-reported and functional outcomes, and costs healthcare systems approximately twice as much as primary surgery.[2,5]

There are currently no established alternative treatments to revision surgery for osteolysis. To date, the most extensively investigated group of drugs explored are the bisphosphonates. Whilst observational studies have associated bisphosphonate use with a lower incidence of prosthesis revision,[6] these findings are not supported by clinical trial data. Rubash et al, in a randomised clinical trial of 123 participants (78 men, 45 women, mean age 63 years) with established femoral osteolytic lesions at 16 centres in the United States, found that daily oral administration of the bisphosphonate alendronate (10mg or 35mg versus placebo) did not affect change in radiological lesion size, visual analogue pain score, or likelihood of progression to revision surgery over 18 months.[7] Animal models that mimic osteolysis have shown that suppression of osteoclast activity through modulation of receptor-activator of NFκB (RANK) signalling inhibits bone resorption and is more effective than bisphosphonates in reducing osteoclast numbers and osteolytic lesion size.[8,9]

Denosumab is a fully human monoclonal antibody with a high affinity for RANK ligand (RANKL) that can bind and neutralize the activity of human RANKL. Denosumab at the 60mg dose has marketing approval in the United Kingdom for the treatment of post-menopausal osteoporosis. In rheumatoid arthritis, a condition also characterised by increased RANKL expression and focal bone erosion, denosumab is also effective in reducing bone erosions yet

bisphosphonates have been ineffective.[10,11] Furthermore, a head-to-head comparison of denosumab versus the bisphosphonate alendronate found the bisphosphonate group to have progression in size of erosions whereas the denosumab group experienced reduced bone erosion size over 6 months following treatment.[12]

In this study, we aimed to assess the effect and short-term safety of a single 60mg dose of denosumab on tissue-specific osteolytic lesion activity in patients with symptomatic, radiographically confirmed osteolysis after THA and who were awaiting revision surgery. Our primary hypothesis was that the group receiving denosumab treatment will have a lower osteoclast number within osteolytic lesions compared to the placebo group. The successful validation of this proof-of-concept would justify phase three trials in participants with earlier-stage disease to test for clinical efficacy in reducing rate of osteolysis progression and the need for revision surgery.

METHODS

Study design and governance

This single-centre, randomised, double-blinded, placebo-controlled, phase two superiority trial was conducted at Sheffield Teaching Hospitals NHS Foundation Trust. Ethical approval for the trial was provided by NRES Committee Yorkshire & The Humber – Leeds West (REC reference 11/YH/0252). We obtained written, informed consent from all participants. The trial was done and analysed according to the protocol that is openly available at http://dx.doi.org/10.17632/gp264xp3rd.1. The trial is registered with the EU Clinical Trials Register (EudraCT 2011-000541-20), and has clinical trial authorisation from the MHRA (21304/0239/001-0001). Sheffield Teaching Hospitals NHS Foundation Trust were the trial sponsor and monitor. They verified adherence to protocol, completeness and accuracy of the data, and that database verification and lock was complete prior to unblinding. The sponsor reviewed all adverse events (reporting to REC and MHRA, as required) and performed ad-hoc visits to verify case record files. A Trial Steering Committee, comprising JMW, RE, and other investigators of the host department reviewed trial conduct at monthly meetings and advised the sponsor in accordance with Good Medical Practice.

Participants

Patients were identified in arthroplasty clinics for possible participation by SCB, AG, AJH, RMK, MWT, and JMW and formally screened for eligibility by the study research nurse

(AGr). Those greater than 30 years of age undergoing revision THA for radiologically-confirmed periprosthetic osteolysis affecting either the femur or pelvis (with or without concurrent prosthesis loosening) and listed for revision surgery were eligible to take part in the trial. Patients with a metal or ceramic on conventional polyethylene bearing were included, as were cemented, hybrid and cementless methods of prosthesis fixation. Patients who had used oral bisphosphonates within the last twelve months or have had greater than three years of cumulative use were not eligible for the study. Any use of intravenous bisphosphonates, fluoride, strontium, parathyroid hormone or its derivatives, anabolic steroids or testosterone, corticosteroids, systemic hormone replacement therapy, selective oestrogen receptor modulator, tibolone, calcitonin or calcitriol were excluded. Patients suffering from hypocalcaemia or having a history of either Paget's disease of the bone, rheumatoid arthritis or malignancy were also not eligible. Patients in whom denosumab is contraindicated along with those who were pregnant, breast feeding or had a known prosthesis infection were also excluded. Recruitment was stopped when 22 participants had completed the trial procedures since this was expected to provide >80% power to meet the primary endpoint.

Randomisation and masking

Following enrolment, participants received a study number and were randomly allocated (1:1) to a single subcutaneous injection either denosumab 60mg or placebo by an independent pharmacist using Documenta Geigy Scientific random number tables, sixth edition. The pharmacist stratified the randomisation in blocks of 10 to produce an equal number of treatment/placebo allocations for up to 30 participants. The dose of 60mg denosumab was selected as a dose-ranging study in rheumatoid arthritis, as a model of inflammatory osteolysis, had previously shown efficacy in trials at this dose on MRI erosion score and modified Sharp erosion score.[13] The treatment allocation was known only to the independent pharmacist. Denosumab (60mg in 1mL solution) and matching placebo were prepared by Amgen Inc, according to the allocation schedule and supplied to the independent pharmacist by unique pack number that mapped to the study number. Both preparations appeared identical apart from the pack number identifier. Participants, investigators, outcome assessors, and care providers were masked to the treatment groups until analysis of the locked trial database following the final study procedures.

Procedures

At the screening baseline visit (-2 weeks) demographic data and a medical history was collected, and blood samples for full blood count, erythrocyte sedimentation rate, C-reactive protein, urea and electrolytes, estimated glomerular filtration rate and serum calcium were taken. Clinical assessments (vital signs, changes to medical history and drugs), patient and clinician-reported outcomes (measured by Oxford[14] and Harris[15] hip scores, respectively), and adverse events were recorded at every visit. (weeks -2, 0, 4, 8, and 14). Blood and urine samples were taken for assessment of biochemical markers of bone turnover at weeks -2, 0, 4, and 8. The bone resorption markers C-telopeptide of type-I collagen (CTX) and tartrate-resistant acid phosphatase 5b (TRAP5b) were measured from serum by Elecsys β-Crosslaps assay (Roche Diagnostics, Indianapolis, IN) and ELISA (Nittobo Medical Ltd, Fukushima, Japan), respectively. The bone resorption markers α-CTX and β-CTX were measured by ELISA (Immunodiagnostic Systems, Ltd, Boldon, UK) from fasting morning urine samples. The bone formation marker total N-terminal propeptide of type-I procollagen (PINP) was measured from serum by Elecsys assay. All assays were performed as a single batch at the end of study following storage at -80°C. The study intervention was administered at visit 2 (week 0). A cone-beam computed tomography scan of the hip was made at the same visit to evaluate the extent of the osteolysis, defined using the American Academy of Orthopaedic Surgery (AAOS) classification system,[16,17] and to determine whether osteolysis involved the pelvis and /or the femur. Participants underwent revision surgery at week 8 (±2 weeks). At surgery, bone involvement and AAOS bone loss grade was confirmed by direct inspection. Representative biopsies of the osteolytic membrane and its underlying bone at the sites of the major osteolytic lesions were taken using a dedicated 6mm internal diameter bone biopsy trephine. The biopsy samples were fixed in 10% neutral buffered formalin prior to decalcification for a minimum of 3 months in EDTA. After decalcification was completed, samples were dehydrated, wax-embedded, sectioned at 4μm and stained for osteoclasts by tartrate-resistant acid phosphatase stain according to standard protocols.

All non-surgical and non-radiological study visit procedures including treatment allocation, were performed by AGr. Radiological procedures were supervised and reported by NH, confirmation of the clinical and radiographic diagnosis was made by JMW, surgical procedures were made by SCB, AG, AJH, RMK, MWT, and JMW. Tissue biopsy sample processing was made by an experienced histology technician (OG) overseen by DH, and biochemical markers of bone turnover were assayed by the same bone biochemistry technician (FG). Histomorphometric measurements were made at x20 magnification on a

Leica DRMB fluorescence microscope (Leica Microsystems, Milton Keynes, UK) using Osteomeasure software (Osteometrics Inc, Atlanta, GA), according to established definitions and methods.[18] All measurements were made in duplicate by MMM. Osteoclasts were defined as TRAP positive cells that stain red with at least one distinct nucleus visible. Only osteoclasts located within one cell distance from the osteolytic membrane-bone interface were counted. Osteoblasts were defined by the presence of a minimum of 3 adjacent cells with at least one osteoblast with a clear eccentric nucleus, cuboidal in shape and plump. Eroded surface was defined as a part of the osteolytic membrane-bone interface in which the bone had been eroded but was osteoclast negative. Quiescent surface was considered the 'inactive' surface and was calculated by subtracting osteoclast surface, osteoblast surface and eroded surface from total osteolytic membrane-bone surface. Areas that did not include osteolytic membrane-bone interface were not measured. The immunohistochemistry slides were prepared by a histology technician (MG) using standard methods and read by OG. For Ki-67, optimised Ki-67 primary antibody diluted 1:400 (Dako, M7240) was applied and incubated overnight at 4C, followed by the secondary antibody in diamino-benzidine chromogen reagent applied for 4 minutes and counterstained with Gill's Hematoxylin. For Caspase 3, polyclonal rabbit Caspase 3 diluted 1:400 (Cell Signalling, Ref-Ab9661) was applied using the same protocol. All slides were dehydrated and mounted with DPX mountant (Sigma, Ref-06522). Human tonsil tissue served as a positive and negative control. The negative control was prepared by omitting the primary antibody.

Outcomes

The primary outcome was the number of osteoclasts per millimetre of osteolytic membrane at the osteolytic membrane-bone interface at week 8 as assessed by static histomorphometry. The secondary outcomes were: The number of osteoblasts per millimetre of membrane, the length of osteoclast surface, eroded surface, and quiescent surface, expressed as a percentage of the total length of osteolytic membrane-bone interface as assessed by static histomorphometry; Percentage cell proliferation and apoptosis throughout the osteolysis membrane as assessed by Ki-67 and Caspase-3 immunostaining, respectively; systemic bone resorption and osteoclast number at weeks -2, 0, 4, and 8 as assessed by serum CTX and TRAP-5b, respectively; Relative resorption rates of newly formed versus mature collagen at weeks -2, 0, 4, and 8 as assessed by the ratio of urinary α-CTX to β-CTX (and corrected for urine concentration by urinary creatinine); And systemic bone formation rate at weeks -2, 0, 4, and 8 as assessed by serum PINP. The safety outcomes were the adverse events recorded at

weeks 0, 4, 8 and 14; and patient and clinician-reported outcomes at weeks 0, 4, 8, and 14, as assessed by Oxford and Harris Hip Score, to identify more subtle potential clinical harms associated with the treatment; and evidence of bone fracture or other prosthesis-related complication at week 14 as assessed by plain radiograph of the hip.

Statistical analysis

The sample size was based on detecting a 50% difference in the absolute number of osteoclasts per millimetre of osteolytic membrane at the osteolytic membrane-bone interface (the primary outcome) between the denosumab group and placebo group (see protocol page 29, http://dx.doi.org/10.17632/gp264xp3rd.1). We chose this effect size to be consistent with the effect of denosumab on osteoclast number in iliac crest biopsies from the STAND and FREEDOM osteoporosis studies,[19] noting that the biological mechanism for the osteoclast activity differs between the diseases and that there are no established histomorphometric standards or minimally important differences for wear-particle-induced osteolysis. The data used to inform the power calculation were taken from counts of osteoclast number in routine histological bone-interface membrane biopsies from archived histology samples in 10 patients with prosthesis-related osteolysis after cemented, hybrid, and cementless THA, and ranging in grade from linear through to expansile osteolysis. Power was estimated by simulation using an estimated mean (standard deviation) osteoclast number of 1·8 (0·6) and 0·9 (0·6) in the control and treatment groups, respectively. At this effect size, a sample of 10, 12 and 15 per group gave a power of 86%, 92% and 97% respectively, assuming a normal distribution and a two-sided alpha of 0·05. In the trial, the study data were not normally distributed for the primary outcome and the primary analysis was performed by Mann-Whitney U test on the median rank between-group difference in osteoclast number in participants who underwent revision surgery at week 8.

All between-group secondary outcomes were analysed by Mann-Whitney U test in participants who underwent revision surgery at week 8. For the bone turnover markers, between-group comparisons were made on the marker change between baseline and week 8, with the baseline value calculated as the mean of the week -2 and week 0 measurement. Within-group biomarker changes between baseline and week 8 were analysed by Wilcoxon test. Bone turnover marker concentrations below the assay detection limit were assigned the lowest quantifiable value within the detectable range of the assay.

Adverse events were analysed between-group by Fisher exact test in all participants who received study drug. The patient and clinician-reported outcomes were analysed by between-group median score change between baseline and the end of the trial (week 14) by Mann-Whitney test in all participants who underwent revision surgery. Between-group differences in hip x-rays at week 14 were analysed qualitatively. All statistical analyses were made two-tailed using SPSS version 25 (IBM, New York, NY). We considered p values of less than 0·05 statistically significant.

Role of the funding source

This study was funded by Amgen, Inc as an investigator-led study. The funder commented on the study design, but had no formal role in its development, nor in the data collection, analysis, interpretation, or writing the report. MMM, RJL and JMW had full access to all the study data and JMW had final responsibility for the decision to submit for publication.

RESULTS

Between December 12, 2012 and June 24, 2018, 51 patients were reviewed for eligibility, of whom 24 were enrolled (Figure 1). Two participants were withdrawn between treatment allocation and surgery for medical fitness reasons unrelated to the study treatment (1 from each treatment group, see adverse events), leaving 10 participants who received 60mg subcutaneous denosumab and 12 who received placebo in the per-protocol analysis. Baseline characteristics of the participants are shown in Table 2. Both baseline demographics and osteolytic lesion distributions were similar between the treatment groups.

For the primary outcome there were 83% fewer osteoclasts at the osteolysis membrane-bone interface (median 0·05/mm [IQR 0·11] versus 0·30/mm [0·40], p=0·011) in the denosumab group versus the placebo group (Figure 2). For the secondary histological outcomes, the osteoclast surface was 87% lower in the denosumab group (0·14% [0·33] versus 1·04% [1·22], p=0·0089), and the eroded surface was 72% lower (0·22% [0·48] versus 0·78% [1·02], p=0·015). The osteoblast number was 90% lower (0·04/mm [0·13] versus 0·41/mm [0·54], p=0·017) and the osteoblast surface was 91% lower (0·05% [0·15] versus 0·53% [0·91], p=0·015) in the denosumab versus placebo group. The most common surface in both treatment groups was the quiescent surface. This surface was 2% greater in the denosumab versus placebo group (99·4% [0·4] versus 97·8% [2·2], p=0·0041). Immunocytochemistry for cell proliferation (Ki67) and apoptosis (Caspase 3) showed no differences between the groups

(appendix p 2, p>0·05). The amount of osteolysis membrane-bone surface interface identified and quantitated was similar in both treatment groups (appendix p 3, p>0·05). The coefficients of variation of the duplicate histomorphometry measurements are shown in appendix p 4.

In the denosumab group there was an acute fall in both the serum and urinary markers of bone resorption after drug administration, reaching a nadir at week 4 that was maintained until revision surgery at week 8 (Figure 3A to 3E). No change in these markers was observed in in the placebo group (between group absolute difference p<0·0003 all biomarkers). No change in the ratio of urinary α:β CTX was observed over the study period, nor any differential change between treatment groups (p=0·31) to suggest a change in the ratio of immature to mature bone resorption induced by the treatment. Following treatment administration a fall in the bone formation PINP was also observed in the denosumab group, reaching 56% at week 8, whilst no significant change was observed in the placebo group (Figure 3F, between group absolute p<0·0001).

Seventeen adverse events were recorded across all enrolled participant over the study period, seven in the denosumab group and ten in the placebo group (p=0·54) and are listed in Table 3. No life-threatening, disabling, or death adverse events were recorded. Two events of arthralgia were reported, and considered possibly related to the drug. One occurred in a denosumab recipient and the other in a placebo recipient. Two participants were withdrawn from the study after treatment allocation and prior to revision surgery. One was withdrawn from the denosumab group for a new diagnosis of oesophageal cancer. The other was withdrawn from the placebo group because they required further anaesthetic assessment prior to surgery that placed them out of study window for the primary outcome. This was not recorded as an adverse event. One participant in the placebo group experienced recurrent dislocations of the hip following revision surgery, requiring a further revision of the acetabular component before the week-14 safety follow up visit.

No significant changes in clinical outcome scores were identified over the study period, except for a modest improvement over the 6 weeks following revision surgery in the placebo group (Appendix p5, p<0.05). No differences in these outcomes between the treatment groups were identified at any time point (p>0·05). The safety follow up radiographs showed no qualitative evidence of prosthesis or bone-related complications in either treatment group,

with the exception of the placebo group participant with recurrent dislocation who underwent second revision prior to the week 14 imaging.

DISCUSSION

To date, there are no pharmacological or biological solutions to the problem of inflammatory osteolysis after joint replacement. Our proof of concept study assessed the effect of the RANKL monoclonal antibody denosumab in reducing osteolytic lesion activity in participants with symptomatic osteolysis after THA. A single 60mg dose of denosumab resulted in a substantial reduction in osteoclast number and other histomorphometric measures of lesion activity within 8 weeks of administration. Denosumab treatment also reduced systemic biochemical markers of bone turnover and was well-tolerated when compared with placebo. The number of adverse events in both study arms was similar, and was consistent with the relatively high morbidity associated with revision joint replacement surgery. The clinical outcomes data was consistent with the denosumab intervention not causing any additional harm.

Several lines of investigation have indicated a central role for RANK signalling in osteolysis. Retrieval studies of interface membranes and hip synovial fluid taken at revision surgery show elevated levels of M-CSF, RANKL and an increased RANKL/ osteoprotegerin (OPG) ratio.[20-22] RANKL mRNA expression in osteolytic lesion interface tissues correlates with lesion size and polyethylene wear volume;[23] and circulating levels of RANKL protein and an increased RANKL/OPG protein ratio are associated with clinical osteolysis.[24] Fibroblasts retrieved from interface membrane and exposed to particle debris express RANKL,[25] and when co-cultured with human monocytes they induce osteoclast formation and lacunar bone resorption.[26] Further, RANK -/- knock-out mice do not develop calvarial osteolysis or increased osteoclast number in response to a particulate stimulus, despite a local inflammatory cellular tissue infiltrate response.[27]

Whilst histomorphometry data from the FREEDOM and STAND studies demonstrated a similar magnitude of suppression of osteoclast number in iliac crest biopsies of patients with post-menopausal osteoporosis,[19] to our knowledge this study represents the first clinical investigation of the effect of this intervention on biological activity within inflammatory osteolytic lesions for which both the initiating stimulus and biological mechanism are different. The histomorphometric and biomarker data indicate suppression of bone turnover

activity at both local and systemic levels by the administered denosumab at the time of biopsy collection. This time point was chosen to coincide with the anticipated maximal efficacy of the drug, as suggested by systemic biomarker data from the FREEDOM trial.[28] The Ki67 and Caspase 3 data further suggest that whilst the denosumab reduced the bone cell count at the membrane surface there was no indication that the drug had an effect on general cellular proliferation or apoptosis within the cell membrane, as these markers are not specific to bone cells.

This direct biological efficacy data provides evidence of effect size to inform power calculations on the numbers of participants required for a phase three study using symptom and lesion progression, and the need for revision surgery as the outcomes. From this data, we estimate that 80 participants randomised at 1:1 ratio would need to be recruited to a study examining the effect of denosumab on change in radiographic lesion size with an alpha of 0.05 and power of 90%. This assumes a conservative 50% suppression in eroded surface that directly corresponds with change in lesion volume, that osteoclast activity accounts for all bone resorption and that the measurement technique used to assess the outcome is sufficiently sensitive.

This study has limitations. The primary criteria for inclusion in the study was wear-particle associated osteolysis in the presence of a polyethylene-containing bearing. Osteolysis occurs around prostheses that are fixed to the adjacent bone with cement and those that are fixed without cement, and thus both fixation methods were represented amongst the participants. We used a single-dose intervention to demonstrate the biological proof of concept by studying patients whose disease was sufficiently severe to require revision surgery and used that opportunity to study the effect of the drug on osteolytic lesions directly. This approach had challenges. The study was slower to recruit than anticipated, as the sample population was a subset of all osteolysis patients, and there was little perceived personal benefit to participation as the primary outcome did not obviate the need for revision surgery. However, the value of direct observation of denosumab on lesion activity provides clear evidence to justify the conduct of further trials examining clinical outcomes. Such studies could be conducted in patients in whom the lesions were less advanced and in whom loosening of the prosthesis was not already established at study enrolment. In order to demonstrate an effect that alters the natural clinical history of the disease, repeated dosing would likely be required, and the clinical outcomes may not directly follow the biological effects nor be sustained.

What are the potential risks of using denosumab in patients with osteolysis? Denosumab has been widely used over the past 10 years for the treatment of postmenopausal osteoporosis. However, there was evidence from the 10-year Phase 3 FREEDOM Trial that there is an increase in the risk of osteonecrosis of the jaw and atypical femur fractures.[29]. The risk of these outcomes relates to duration of treatment. In the FREEDOM trial, none of the 13 cases of osteonecrosis of the jaw and none of the two cases of atypical femur fracture occurred within the first three years of therapy. There is also evidence for a period of accelerated bone loss in the first year of stopping denosumab therapy that is likely the result of high bone turnover and the consequence is an increase in the risk of multiple vertebral fractures.[30] Their relevance to the dosing, duration and route of administration that may be required in the management of periprosthetic osteolysis is yet to be determined.

In conclusion, the results of this proof-of-concept clinical trial indicate that denosumab is effective at reducing bone resorption activity within osteolytic lesion tissue and is well-tolerated within the limitations of the single dose used here. Given this demonstration of biological effect, phase three studies are warranted to examine the clinical efficacy of denosumab in halting the progression of osteolytic lesions using clinical outcome as the measure of efficacy.

Contributors

JMW and DH designed the study. SCB, AG, AJH, MWT, RMK and JMW contributed to participant identification. All authors contributed to the clinical evaluations and/or data collection. MMM and JMW performed the literature searches. MMM, RJL and JMW verified the underlying data. MMM and RLJ did the statistical analysis. MMM and JMW wrote the first draft of the article. All authors interpreted the data, contributed to writing, critically revised the article, and approved the final submitted version.

Declarations of interest

RE declares grants from Alexion, Amgen, IDS, Roche, Nittobo, and personal fees from Amgen, IDS, Roche, GSK Nutrition, Mereo, Sandoz, Nittobo, AbbVie, Samsung, Haoma Medica, Elsevier, CL Bio, FNIH, Viking, UCSF, Biocon and Lyramid outside the submitted work. All other authors declare funding from Amgen for this study but no other competing interest.

Data sharing statement

The individual deidentified participant level data for this study, together with the codes defining each field and the study protocol are openly available at Wilkinson, Jeremy (2020), "EudraCT 2011-000541-20 participant level data", Mendeley Data, v1 http://dx.doi.org/10.17632/gp264xp3rd.1

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LEGEND TO FIGURES

Figure 1: Trial profile

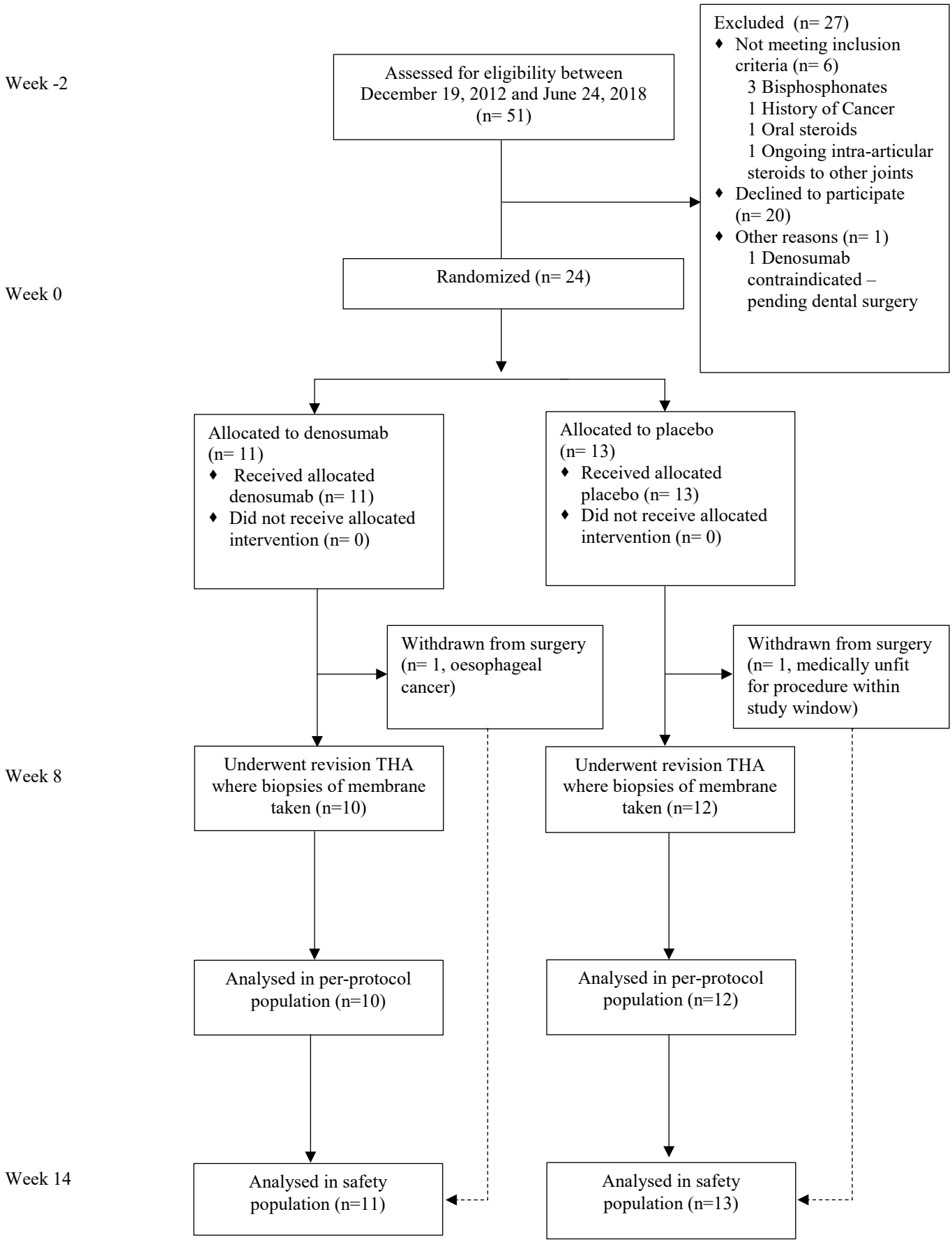
Figure 2: Histomorphometric outcomes. Truncated violin plots showing individual datapoints, coloured dashed error bar is median and black dotted error bars show interquartile range. Analysis is between-group by Mann-Whitney U test of ranks.

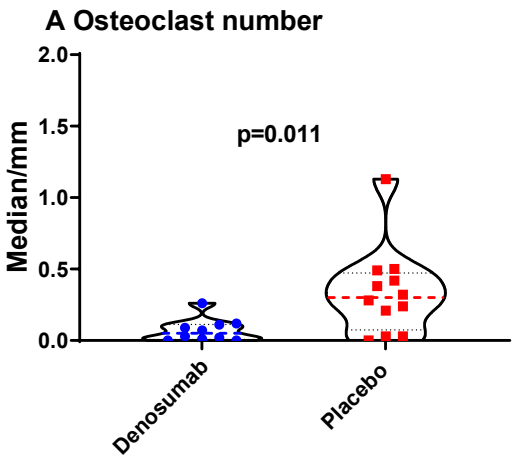
Figure 3: Bone turnover marker outcomes. Absolute median and interquartile range values for each marker are show in top panel, together with analysis of within-group changes versus baseline by Wilcoxon test. Bottom panel shows the estimated difference (ED) of denosumab minus placebo medians and 95% confidence interval at each time point, using the Hodges Lehmann estimator. Between-group comparisons were made on the absolute marker change by Mann-Whitney U test of ranks, with the baseline value calculated as the mean of the week -2 and week 0 measurement.. Only significant p-values are shown. Arrow shows drug administration point.

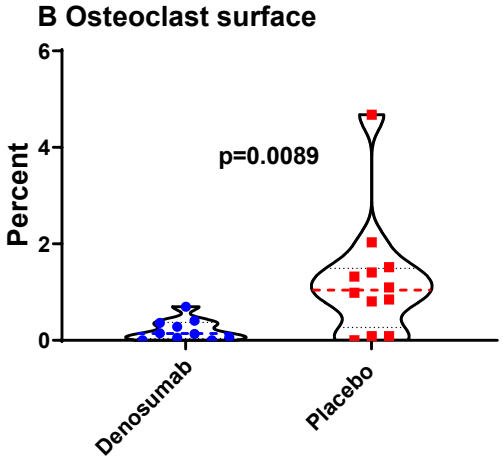
Table 1. Indication for revision hip replacement surgery across large publically reported European, Australasian and Canadian joint replacement registries.

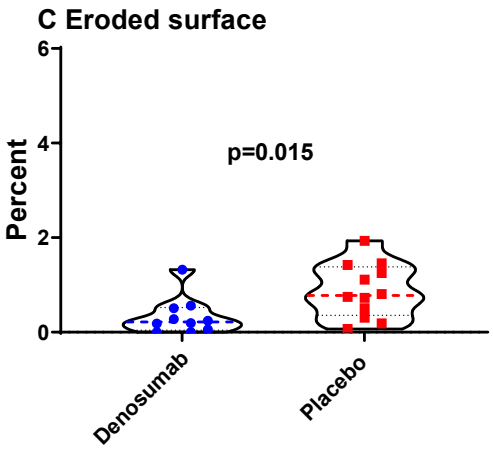
†Single-stage revisions only, indications are not mutually exclusive; ††figures for aseptic loosening and osteolysis (without loosening) combined;*absolute number calculated from percentage figure given in report.

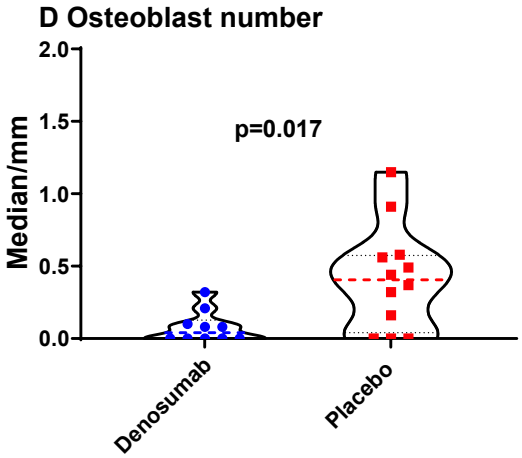
Register coverage	Report year	Report Period	Number of revisions in period	Most frequent revision indication††	2nd most frequent revision indication	3rd most frequent revision indication	Web link to report
England & Wales†	2019	Apr 2003 - Dec 2018	101,012	Osteolysis/loosening n=63,670 (63%)	Pain n=18,100 (18%)	Dislocation n=16,091 (16%)	https://reports.njrcentre.org.uk/Portals/0/PDFdownloads/NJR%2016th%20Annual%20Report%202019.pdf
England & Wales†	2019	Jan 2014 - Dec 2018	38,550	Osteolysis/loosening n=20,646 (53%)	Dislocation n=6,699 (17%)	Periprosthetic fracture n=5,637 (15%)	https://reports.njrcentre.org.uk/Portals/0/PDFdownloads/NJR%2016th%20Annual%20Report%202019.pdf
Norway	2019	Jan 2018 - Dec 2018	2,028	Osteolysis/loosening n=721 (36%)	Infection n=372 (18%)	Dislocation n=271 (13%)	http://nrlweb.ihelse.net/eng/Rapporter/Report2019_english.pdf
Sweden	2018	Jan 2016 - Dec 2018	6,848	*Osteolysis/loosening n=2,465 (36%)	*Infection n=1986 (29%)	*Dislocation n=959 (14%)	https://registercentrum.blob.core.windows.net/shpr/r/Arsrapport_2018_Hoftprotes_ENG_26mars_Final-rJepCXNsLI.pdf
Denmark	2019	Jan 2018 - Dec 2018	884	Osteolysis/loosening n=253 (29%)	Dislocation n=206 (23%)	Periprosthetic fracture n=145 (16%)	http://danskhoftealloplastikregister.dk/wp-content/uploads/2019/09/DHR-årsrapport-2019_til-offentliggørelse-1.pdf
Germany	2019	Jan 2018 - Dec 2018	14,653	Osteolysis/loosening n=4,498 (31%)	Infection n=2,197 (15%)	Dislocation n=1,758 (12%)	https://www.eprd.de/fileadmin/user_upload/Dateien/Publikationen/Berichte/EPRD_Jahresbericht_2019_EN_doppelseitig_F_Web.pdf
Netherlands	2019	Jan 2018 - Dec 2018	3,788	Osteolysis/loosening n=1,530 (40%)	Infection n=780 (21%)	Dislocation n=716 (19%)	https://www.lroi-rapportage.nl/media/pdf/PDF%20Online%20LROI%20annual%20report%202019.pdf
Australia	2019	Jan 2003 - Dec 2018	70,730	Osteolysis/loosening n=30,473 (43%)	Infection n=11,623 (16%)	Dislocation n=10,191 (14%)	https://aoanjrr.sahmri.com/documents/10180/671402/Revision+Hip+and+Knee+Arthroplasty
New Zealand	2019	Jan 2018 - Dec 2018	629	Osteolysis/loosening n=213 (34%)	Dislocation n=101 (16%)	Infection n=97 (15%)	https://nzoa.org.nz/system/files/DH8328_NZJR_2019_Report_v4_7Nov19.pdf
Canada	2019	Jan 2017 - Dec 2018	4,822	*Osteolysis/loosening n=1,206 (25%)	*Dislocation n=868 (18%)	*Infection n=868 (18%)	https://secure.cihi.ca/free_products/cjrr-annual-report-2019-en-web.pdf

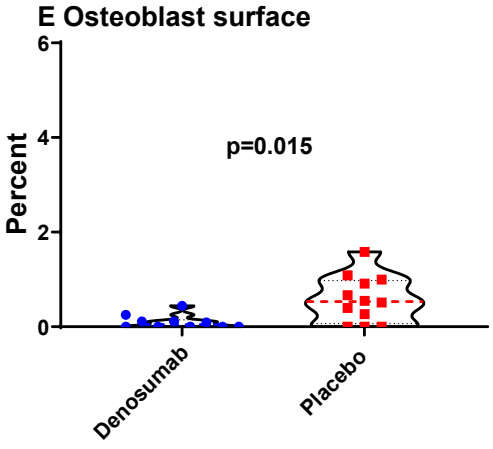

Week -2
Assessed for eligibility between December 19, 2012 and June 24, 2018 (n= 51)
Excluded (n= 27)
♦ Not meeting inclusion criteria (n= 6)
3 Bisphosphonates
1 History of Cancer
1 Oral steroids
1 Ongoing intra-articular steroids to other joints
♦ Declined to participate (n= 20)
♦ Other reasons (n= 1)
1 Denosumab contraindicated – pending dental surgery
Week 0
Randomized (n= 24)
Allocated to denosumab (n= 11)
♦ Received allocated denosumab (n= 11)
♦ Did not receive allocated intervention (n= 0)
Allocated to placebo (n= 13)
♦ Received allocated placebo (n= 13)
♦ Did not receive allocated intervention (n= 0)
Withdrawn from surgery (n= 1, oesophageal cancer)
Withdrawn from surgery (n= 1, medically unfit for procedure within study window)
Week 8
Underwent revision THA where biopsies of membrane taken (n=10)
Underwent revision THA where biopsies of membrane taken (n=12)
Analysed in per-protocol population (n=10)
Analysed in per-protocol population (n=12)
Week 14
Analysed in safety population (n=11)
Analysed in safety population (n=13)

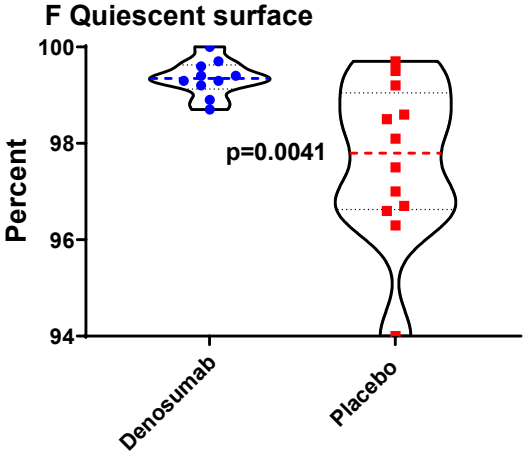

A Osteoclast number
Median/mm
2.0
1.5
1.0
0.5
0.0
p=0.011
Denosumab
Placebo

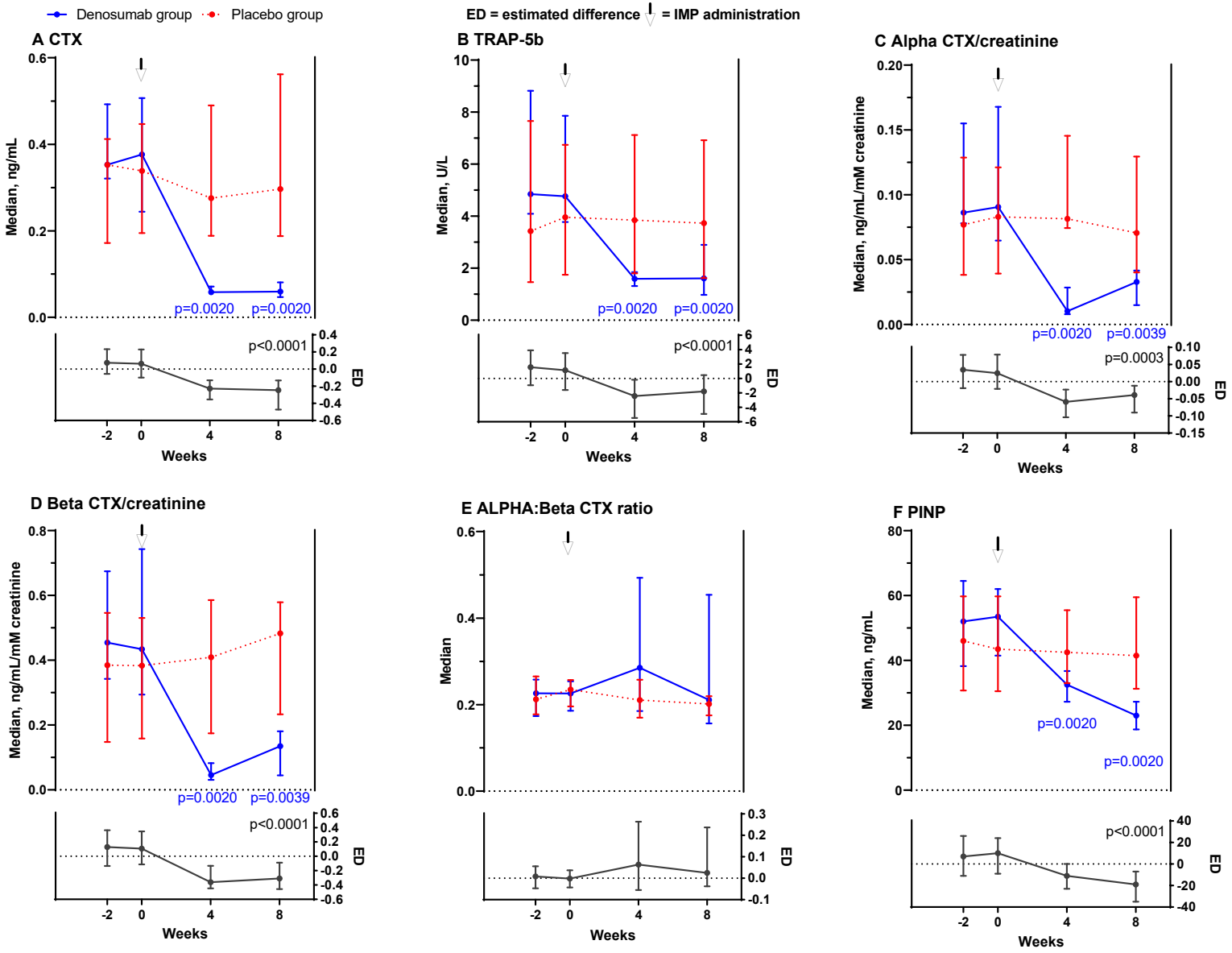

B Osteoclast surface
Percent
6
4
2
0
p=0.0089
Denosumab
Placebo


C Eroded surface
Percent
6
4
2
0
p=0.015
Denosumab
Placebo

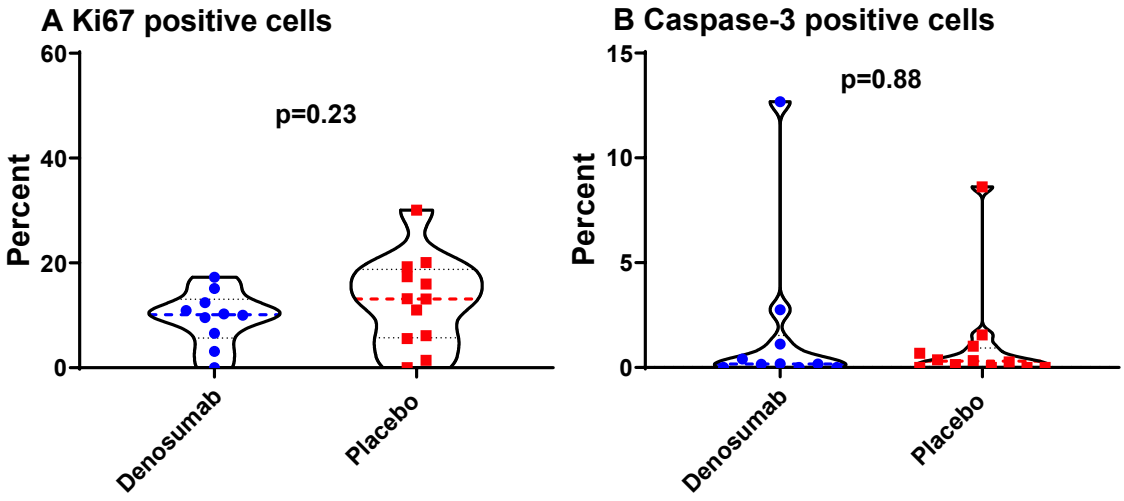

D Osteoblast number
Median/mm
2.0
1.5
1.0
0.5
0.0
p=0.017
Denosumab
Placebo


E Osteoblast surface
Percent
6
4
2
0
p=0.015
Denosumab
Placebo

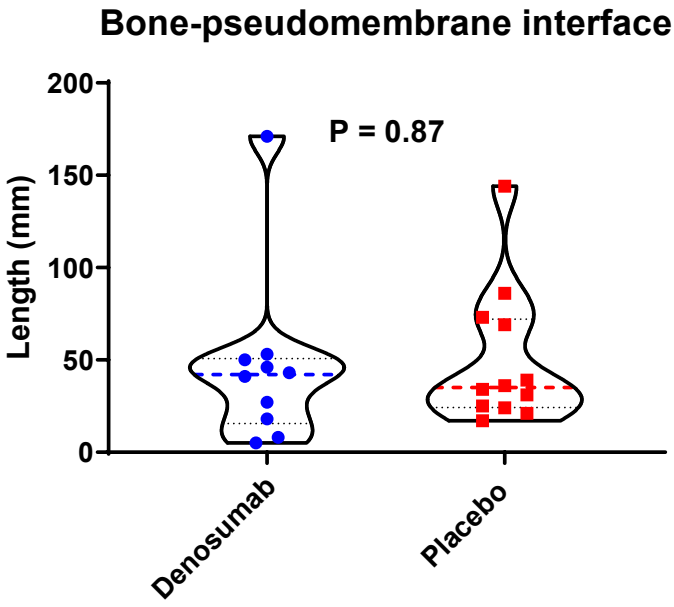

F Quiescent surface
Percent
100
98
96
94
p=0.0041
Denosumab
Placebo


Denosumab group
Placebo group
ED = estimated difference
= IMP administration
A CTX
Median, ng/mL
p=0.0020
p=0.0020
p<0.0001
ED
Weeks
B TRAP-5b
Median, U/L
p=0.0020
p=0.0020
p<0.0001
ED
Weeks
C Alpha CTX/creatinine
Median, ng/mL/mM creatinine
p=0.0020
p=0.0039
p=0.0003
ED
Weeks
D Beta CTX/creatinine
Median, ng/mL/mM creatinine
p=0.0020
p=0.0039
p<0.0001
ED
Weeks
E ALPHA:Beta CTX ratio
Median
ED
Weeks
F PINP
Median, ng/mL
p=0.0020
p=0.0020
p<0.0001
ED
Weeks

Appendix Figure: Immunohistochemistry outcomes. Truncated violin plots showing individual datapoints, coloured dashed error bar is median and black dotted error bars show interquartile range. Analysis is between-group by Mann-Whitney U test of ranks.



Appendix Figure: Length of bone-pseudomembrane interface measured in each participant group. Truncated violin plots showing individual datapoints, coloured dashed error bar is median and black dotted error bars show interquartile range. Analysis is between-group by Mann-Whitney U test of ranks.



Appendix Table - Intra-observer variation in histomorphometric measurements. All outcomes were measured independently by the same observer with a gap of at least 2 weeks between measurements

Histomorphometry Indices	**Coefficient of Variation (%)**
Osteolytic membrane-bone interface	3·1
Number of osteoclasts/membrane (mm)	7·0
Number of osteoblasts/membrane (mm)	10·7
Osteoclast surface (%)	15·7
Osteoblast surface (%)	21·4
Eroded surface (%)	55·9
Quiescent surface (%)	0·4

Appendix Figure: Patient and clinician-reported safety outcomes. Absolute median and interquartile range values for each measure are show in top panel, together with analysis of within-group changes versus baseline by Wilcoxon test. Bottom panel shows the estimated difference (ED) of denosumab minus placebo medians and 95% confidence interval at each time point, using the Hodges Lehmann estimator. Between-group comparisons were made on the absolute marker change by Mann-Whitney U test of ranks. Only significant p-values are shown. Arrow shows drug administration point.