

## Effect of Denosumab or Alendronic Acid on the Progression of Aortic Stenosis: A Double-Blind Randomized Controlled Trial

**Running Title:** Pawade & Doris, et al.; Denosumab or Alendronic Acid for Aortic Stenosis

Tania A. Pawade, MD<sup>1\*</sup>; Mhairi K. Doris, MD<sup>1\*</sup>; Rong Bing, MBBS<sup>1\*</sup>; Audrey C. White<sup>1</sup>; Laura Forsyth, PhD<sup>2</sup>; Emily Evans, MSc<sup>3</sup>; Catriona Graham, MSc<sup>3</sup>; Michelle C. Williams, MD<sup>1</sup>; Edwin J.R. van Beek, MD<sup>4</sup>; Alison Fletcher, PhD<sup>2</sup>; Philip D. Adamson, MBChB<sup>1,5</sup>; Jack P.M. Andrews, MD<sup>1</sup>; Timothy R.G. Carlidge, MD<sup>1</sup>; William S.A. Jenkins, MD<sup>1</sup>; Maaz Syed, MD<sup>1</sup>; Takeshi Fujisawa, PhD<sup>1</sup>; Christophe Lucatelli, PhD<sup>4</sup>; William Fraser, MBChB<sup>6</sup>; Stuart H. Ralston, MD<sup>7</sup>; Nicholas Boon, MD<sup>1</sup>; Bernard Prendergast, MD<sup>8</sup>; David E. Newby, MD<sup>1</sup>; Marc R. Dweck, MD<sup>1</sup>

<sup>1</sup>BHF Centre for Cardiovascular Science, University of Edinburgh, Edinburgh, UK; <sup>2</sup>Edinburgh Clinical Trials Unit, University of Edinburgh, Edinburgh, UK; <sup>3</sup>Edinburgh Clinical Research Facility, University of Edinburgh, Edinburgh, UK; <sup>4</sup>Edinburgh Imaging, University of Edinburgh, Edinburgh, UK; <sup>5</sup>Christchurch Heart Institute, University of Otago, Christchurch, New Zealand; <sup>6</sup>Norwich Medical School, University of East Anglia, Norwich, UK; <sup>7</sup>Institute of Genetics and Molecular Medicine, University of Edinburgh, UK; <sup>8</sup>King's College London, London, UK



### Address for Correspondence

Marc Dweck, MD  
BHF Centre for Cardiovascular Science  
University of Edinburgh  
47 Little France Crescent  
Edinburgh EH16 4TJ  
Email: [marc.dweck@ed.ac.uk](mailto:marc.dweck@ed.ac.uk)

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## Abstract

**Background:** Valvular calcification is central to the pathogenesis and progression of aortic stenosis, with pre-clinical and observational studies suggesting that bone turnover and osteoblastic differentiation of valvular interstitial cells are important contributory mechanisms. We aimed to establish whether inhibition of these pathways with denosumab or alendronic acid could reduce disease progression in aortic stenosis.

**Methods:** In a single-centre parallel group double-blind randomized controlled trial, patients over 50 years of age with calcific aortic stenosis (peak aortic jet velocity  $>2.5$  m/s) were randomized 2:1:2:1 to denosumab (60 mg every 6 months), placebo injection, alendronic acid (70 mg once weekly) or placebo capsule. Participants underwent serial assessments with Doppler echocardiography, computed tomography aortic valve calcium scoring and  $^{18}\text{F}$ -sodium fluoride positron emission tomography and computed tomography. The primary endpoint was the calculated 24-month change in aortic valve calcium score.

**Results:** One-hundred and fifty patients (mean age  $72\pm 8$  years; 21% female) with calcific aortic stenosis (peak aortic jet velocity  $3.36$  [ $2.93$  to  $3.82$ ] m/s; aortic valve calcium score  $1152$  [ $655$  to  $2065$ ] Agatston Units) were randomized and received the allocated trial intervention: denosumab ( $n=49$ ), alendronic acid ( $n=51$ ) and placebo (injection  $n=25$ , capsule  $n=25$ ; pooled for analysis). Serum C-terminal telopeptide, a measure of bone turnover, halved from baseline to 6 months with denosumab ( $0.23$  [ $0.18$  to  $0.33$ ] to  $0.11$  [ $0.08$  to  $0.17$ ]  $\mu\text{g/L}$ ) and alendronic acid ( $0.20$  [ $0.14$  to  $0.28$ ] to  $0.09$  [ $0.08$  to  $0.13$ ]  $\mu\text{g/L}$ ) but was unchanged with placebo ( $0.23$  [ $0.17$  to  $0.30$ ] to  $0.26$  [ $0.16$  to  $0.31$ ]  $\mu\text{g/L}$ ). There were no differences in 24-month change in aortic valve calcium score between denosumab and placebo ( $343$  [ $198$  to  $804$ ] AU *versus*  $354$  [ $76$  to  $675$ ] AU,  $p=0.41$ ), or alendronic acid and placebo ( $326$  [ $138$  to  $813$ ] AU *versus*  $354$  [ $76$  to  $675$ ] AU,  $p=0.49$ ). Similarly, there were no differences in change in peak aortic jet velocity or  $^{18}\text{F}$ -sodium fluoride aortic valve uptake.

**Conclusions:** Neither denosumab nor alendronic acid affected progression of aortic valve calcification in patients with calcific aortic stenosis. Alternative pathways and mechanisms need to be explored to identify disease-modifying therapies for the growing population of patients with this potentially fatal condition.

**Clinical Trial Registration:** <https://www.clinicaltrials.gov> Unique Identifier: NCT02132026.

**Key Words:** denosumab; alendronic acid; aortic stenosis; computed tomography; calcium score

### Non-standard Abbreviations and Acronyms

18F-NaF	18F-sodium fluoride
AU	Agatston Units
CI	confidence interval
CT	computed tomography
OPG	osteoprotegerin
PET	positron emission tomography
RANK	receptor activator of nuclear kappa B
RANKL	receptor activator of nuclear kappa B ligand
SALTIRE2	Study Investigating the Effect of Drugs Used to Treat Osteoporosis on the Progression of Calcific Aortic Stenosis
SEAS	Simvastatin and Ezetimibe in Aortic Stenosis

$SUV_{max}$	maximum standardised uptake value
$SUV_{mean}$	mean standardised uptake value
$TBR_{max}$	maximum target to background ratio
$TBR_{mean}$	mean target to background ratio

## Clinical Perspective

### What is new?

- Active calcification in the aortic valve has been recognised to be a major determinant of disease progression in aortic stenosis.
- This is the first double-blind randomized controlled trial to test whether drugs targeting processes of active calcification - denosumab or alendronic acid - could slow the progression of aortic stenosis.
- We found that denosumab and alendronate have no major effect on the progression of aortic stenosis as assessed by echocardiography, computed tomography or  $^{18}F$ -sodium fluoride positron emission tomography.

### What are the clinical implications?

- Neither denosumab nor alendronate cause major amelioration or acceleration of aortic valve calcification or disease progression.
- Other pathways need to be explored in order identify an effective therapy for this unmet clinical need.

## Introduction

Despite decades of research and several randomized controlled trials<sup>1-3</sup>, aortic stenosis remains a disease without an effective medical treatment. Prolonged longevity and the subsequent aging of the general population means that the prevalence of aortic stenosis continues to rise. This has led to the increased use of valve replacement interventions which remain the only treatment for end-stage disease, although they carry potentially significant peri-procedural and long-term risks<sup>4,5</sup>. A medical therapy that slows the progression of aortic stenosis would therefore be a major advance that addresses an important unmet clinical need.

The pathology of aortic stenosis is driven by actively regulated inflammation and calcification and has clear similarities to skeletal bone formation<sup>6-10</sup>. Activity of both osteoblasts in the valve and osteoclasts in the bone appears to be important, with multiple potential pathways involved in regulating bone turnover and valvular calcification<sup>9,11</sup>. The receptor activator of nuclear kappa B (RANK) ligand (RANKL)/RANK/osteoprotegerin (OPG) axis is one such pathway. In the valve, RANKL binding stimulates osteogenic differentiation of valvular interstitial cell into osteoblasts, leading to formation of calcific nodules and expression of alkaline phosphatase and osteocalcin. In the bone, RANKL binding stimulates osteoclastic activity, causing release of calcium and phosphate into the cardiovascular system. In murine models, targeted inactivation of OPG, a decoy receptor for RANKL, leads to widespread vascular calcification and severe osteoporosis which can be rescued by administration of osteoprotegerin<sup>12,13</sup>. Pre-clinical studies also demonstrate that bisphosphonates can reduce production of pro-inflammatory cytokines, decrease bone osteoclastic activity and inhibit arterial as well as valvular calcification<sup>14</sup>. Mechanistic clinical data have demonstrated a link between aortic stenosis progression, vitamin D and bone re-modelling<sup>15</sup>, while observational clinical

studies have demonstrated an association between bisphosphonate use and reduced aortic stenosis progression and coronary calcification<sup>16-18</sup>. Further support comes from the Multi-Ethnic Study of Atherosclerosis registry which found that bisphosphonate use was associated with a lower prevalence of cardiovascular calcification (defined as the prevalence of aortic valve, aortic valve ring, mitral annulus, thoracic aorta, and coronary artery calcification on computed tomography [CT]) in women over 65 years of age<sup>19</sup>. However, such associations are not a universal finding<sup>20</sup>, and a causal relationship cannot be established without randomized controlled trial data.

We have demonstrated the active nature of aortic valve degeneration with *in vivo* 18F-sodium fluoride (18F-NaF) positron emission tomography (PET)-CT<sup>21</sup>. 18F-NaF is a bone tracer that binds to hydroxyapatite, a key crystalline component of valvular calcification which has a greater surface area in regions of developing microscopic calcification. Higher valvular 18F-NaF uptake is independently associated with more rapid disease progression and therefore represents a potential biomarker of aortic stenosis disease activity<sup>22</sup>.

On the basis of these data, we conducted the Study Investigating the Effect of Drugs Used to Treat Osteoporosis on the Progression of Calcific Aortic Stenosis (SALTIRE2) randomized controlled trial to determine whether the RANKL inhibitor, denosumab, or the bisphosphonate, alendronic acid, could reduce disease progression in patients with calcific aortic stenosis.

## Methods

### Trial Design and Population

This was a single centre parallel group double blind randomized controlled trial. The Trial Steering Committee oversaw the conduct and progress of the trial. All patients provided written informed consent. The study was conducted in accordance with the Declaration of Helsinki, approved by the regional ethics committee (Scotland A Research Ethics Committee, 14/SS/0064) and registered on clinicaltrials.gov (NCT02132026). The study data are not currently in a public repository but may be made available to researchers upon reasonable request.

Patients over 50 years of age with a peak aortic jet velocity  $>2.5$  m/s on Doppler echocardiography and grade 2-4 aortic valve calcification on semi-quantitative echocardiographic assessment<sup>23</sup> were identified from cardiology outpatient clinics across the south east of Scotland: Edinburgh Heart Centre, Borders General Hospital, Victoria Hospital, Ninewells Hospital and Forth Valley Royal Hospital. Exclusion criteria were anticipated or planned aortic valve surgery in the next 6 months, life expectancy  $<2$  years, inability to undergo scanning, treatment for osteoporosis with bisphosphonates or denosumab, long-term corticosteroid use, abnormalities of the oesophagus or conditions which delay gastric emptying, inability to sit or stand for at least 30 minutes, known allergy or intolerance to alendronic acid, denosumab or any of their excipients, hypocalcemia, regular calcium supplementation, dental extraction within 6 months, history of osteonecrosis of the jaw, major or untreated cancers, poor dental hygiene, women of child-bearing potential who had experienced menarche, were premenopausal, had not been sterilised or were pregnant, women who were breastfeeding, chronic kidney disease (estimated glomerular filtration rate of  $<30$  mL/min/1.73 m<sup>2</sup>), allergy or

contraindication to iodinated contrast, inability or unwillingness to give informed consent, or a likelihood of non-compliance to treatment allocation or study protocol.

### **Trial Protocol**

Participants underwent clinical history and examination, 6-minute walk test, blood sampling, 12-lead electrocardiogram, echocardiography, combined 18F-NaF PET-CT and non-contrast CT. Participants were randomized using computer-based randomization (Edinburgh Clinical Trials Unit, University of Edinburgh) to ensure allocation concealment 4 to 8 weeks after the baseline visit. Patients were allocated to one of four groups - subcutaneous denosumab (Prolia, Amgen, CA) 60 mg every 6 months, placebo injection every 6 months, oral alendronic acid (TEVA UK, UK) 70 mg once weekly or matching placebo capsule once weekly - in a 2:1:2:1 ratio using a minimization algorithm that incorporated a random component. Minimization criteria were age (<73 and  $\geq$ 73 years), sex, presence or absence of a bicuspid valve and baseline aortic valve calcium scores ( $\leq$ 1607 and  $>$ 1607 Agatston units [AU]). Participants were randomized in advance of their randomization visit in order to ensure that the study drug was available to be dispensed at the visit. The placebo capsule contained lactose monohydrate and was manufactured by the Investigational Supplies Group (University of Edinburgh) to be indistinguishable from the encapsulated alendronic acid used. The placebo injection was 0.9% saline, with drug preparation and administration for the injection undertaken by a nominated group of research nurses who remained unblinded. The injection syringes were masked to ensure patients and the research team remained blinded to treatment allocation. Compliance was calculated as the proportion of expected treatment received for the duration of study participation. For participants in the capsule arms, 32 capsules were given to the participant at each study visit (6-month intervals),

and any unused capsules were returned at the subsequent visit, with the assumption that any unreturned capsules were taken as prescribed.

A telephone visit was undertaken 2 weeks after randomisation to assess for symptoms of hypocalcemia. Further study follow-up visits were performed at 6, 12, 18 and 24 months, where clinical examination, electrocardiogram, echocardiogram and blood sampling were undertaken. Serum C-terminal telopeptide, a marker of bone resorption, was measured at baseline and 6 months. Repeat <sup>18</sup>F-NaF PET-CT and non-contrast CT were performed at 12 months and repeat non-contrast CT performed at 24 months. Where possible, participants who were subsequently scheduled for aortic valve replacement had their pending 12- or 24-month visit brought forward, after which the trial intervention was discontinued, and no further trial imaging was performed.

## **Trial Procedures**



### *Echocardiography*

All study echocardiograms were performed by a single dedicated research ultrasonographer (AW) or cardiology research fellow (TAP) on the same echocardiography machine, in the same accredited department using a standardised protocol according to international guidelines.

Standard 2-dimensional views and pulsed and continuous wave Doppler measurements were acquired, with Doppler measurements averaged over three cardiac cycles, or five if the patient was in atrial fibrillation<sup>24</sup>. Aortic valve mean pressure gradient was calculated using the Bernoulli equation. Aortic valve area was estimated using the continuity equation. Aortic stenosis was categorised using standard definitions for peak velocity (mild: 2.6-2.9, moderate: 3.0-4.0, severe: >4.0 m/s) and mean gradient (mild: <20, moderate: 20-40, severe: >40 mmHg)<sup>25</sup>. Ejection fraction was visually estimated and categorised as normal ( $\geq 55\%$ ), mildly impaired (45-54%), moderately impaired (36-44%) or severely impaired ( $\leq 35\%$ )<sup>26</sup>.



*Non-contrast CT and Combined PET-CT*

Unless contraindicated, intravenous or oral metoprolol was administered to patients with a heart rate >65 /min. Imaging was performed on a 128-multislice scanner (Biograph mCT, Siemens, Germany) in a dedicated research imaging centre (Edinburgh Imaging Facility, University of Edinburgh). Non-contrast CT was performed at baseline, 12 months and 24 months using the same scanner, electrocardiogram gating and a standardised protocol (120kV CARE Dose4D [Siemens], 3-mm slice thickness, spiral acquisition, 70% R-R interval, inspiratory breath-hold). 18F-NaF PET-CT was performed on the same scanner at baseline and 12 months. PET image acquisition was performed approximately 60 min after intravenous injection of 125 MBq 18F-NaF with a single bed position centred on the aortic valve. Intravenous iodinated contrast (80 mL Iomeron-400, Bracco Imaging, Italy) was given following PET acquisition, followed by prospective electrocardiogram-gated contrast CT acquisition in diastole (CARE Dose4D, Siemens; 0.75-mm slice thickness, spiral acquisition, 50-75% R-R interval, expiratory breath-hold). Image analysis was performed using Vitrea v6.9.68.1 (Vitrea Advanced, Vital Images, Minnetonka, USA) and FusionQuant v1.20.05.14 (Cedars-Sinai, CA, USA). Aortic valve calcium scores were measured using a standardised technique<sup>27</sup>, with regions of interest drawn around areas of valvular calcification on sequential axial slices. Care was taken to exclude calcification in adjacent structures such as the left ventricular outflow tract or sinuses of Valsalva. A standard threshold of 130 Hounsfield units was used to define calcification. The Agatston score was semi-automatically calculated by the software using standard weightings<sup>28</sup>. 18F-NaF aortic valve uptake was measured using a standardised technique of valve orientation *en face* following co-registration of PET and contrast CT based on blood pool uptake in the cardiac chambers<sup>29, 30</sup>. The valve region of interest was defined by a polyhedron 6-mm in height,

centred on the valvular region of highest visual uptake in the  $z$  plane and contoured manually around the valve perimeter. Blood pool activity was calculated from a 2-cm<sup>2</sup> region drawn at the centre of the right atrium at the level of the right coronary artery ostium. The mean and maximum target to background ratios ( $TBR_{\text{mean}}$  and  $TBR_{\text{max}}$ ) were calculated by dividing the mean and maximum standardised uptake values ( $SUV_{\text{mean}}$  and  $SUV_{\text{max}}$ ) in the region of interest by the mean blood pool standardised uptake value. These measures have excellent reproducibility<sup>24, 29, 31</sup>.

### **Trial Endpoints**

The primary endpoint was the calculated change in aortic valve calcium score at 24 months. Key secondary endpoints included change in peak aortic jet velocity at 24 months and change in aortic valve uptake at 12 months. The primary endpoint was calculated as follows: [(final visit aortic valve calcium score – baseline visit aortic valve calcium score) / days from baseline visit to final visit] \* 730. Where the participant did not attend a 24-month visit but did attend a 12-month visit, the 12-month visit was used as the final visit. Other imaging endpoints were calculated in the same way. In the case of endpoints with 12-month change (aortic valve 18F-NaF uptake), the daily rate of change was multiplied by 365 rather than 730.

### **Statistical Analysis**

To be clinically meaningful, we posited that a disease-modifying therapy would need to delay the time to surgery by 1-2 years. In the previous Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) trial<sup>2</sup>, ~40% of trial participants either died (11%) or underwent aortic valve replacement (30%) within 4 years with an overall rate of disease progression of 0.61±0.59 m/s. This suggests we would need to see a difference in the rates of disease progression of approximately 40% (from 0.16 to 0.10 m/s/year) to delay the need for surgery by 1-2 years.

Based on aortic valve calcium score progression in participants from our prior studies<sup>1,22</sup> who had an aortic valve calcium score  $\geq 400$  (median 2-year change 565 [interquartile range, 190-910] AU), we calculated that a sample size of 47 participants would be required per group to detect a 40% difference in the primary endpoint with a two-sided 5% level of significance and 80% power. To account for missing data, the total study sample size was increased to 150. For the primary endpoint and secondary imaging endpoints, 24- or 12-month change was calculated based upon a daily rate of change from baseline to the relevant follow-up scan, using an intention to treat analysis regardless of compliance. Both placebo groups were combined for analysis. For the primary endpoint, if the baseline non-contrast CT scan was degraded by artefact but 12- and 24-month scans were available, these scans were used to determine the daily rate of change. Sensitivity analyses for the primary endpoint were performed after excluding scans with artefact or incorporating only those participants with at least 50% or 70% compliance.

Categorical variables are presented as number (%) while continuous variables are presented as median [interquartile range] or mean  $\pm$  standard deviation. Distributions of data were tested for normality with the Shapiro-Wilk test and quantile-quantile plots. Between-group differences were compared with the Wilcoxon rank sum test or Kruskal-Wallis test as appropriate. To take into account repeated measurements, mixed-effects linear regression models were constructed for each treatment arm with aortic valve calcium score as the dependent variable, study arm and timepoint as fixed effects and participant as a random effect. Least square means for each active trial arm model were calculated and compared with placebo separately. Spearman's rank correlation coefficient was performed to assess the relationship between continuous variables. Analysis was performed using SAS Enterprise Guide v 7.15 (SAS

Institute Inc., Cary, NC, USA). A two-sided p value of  $<0.05$  was considered statistically significant.

## Results

### Trial Population

Between 19<sup>th</sup> August 2015 and 6<sup>th</sup> November 2017, 199 patients were consented, of whom 152 were randomised to denosumab, alendronic acid or matched placebo. Two participants who were randomized to denosumab and were unaware of their study allocation did not attend the randomization visit or participate further in the study, leaving 150 participants for inclusion in the final analysis (Figure 1).

Baseline characteristics were balanced between study arms (Table 1). The mean age was  $72\pm 8$  years, 21% of the cohort were female, and most were of white Scottish ethnicity. There was a high prevalence of hypertension and hypercholesterolemia. The median aortic valve peak velocity and mean gradient were 3.36 [2.93 to 3.82] m/s and 23 [18 to 32] mmHg respectively.

The median aortic valve calcium score was 1152 [655 to 2065] AU (Table 1).

### Trial Intervention

Compliance was similar between placebo and active drug for each method of administration (proportion of participants receiving  $>70\%$  expected dose: denosumab 94%, placebo injection 92%, alendronic acid 88%, placebo capsule 84%). Baseline serum C-terminal telopeptide concentrations were similar between treatment arms (Table 1) and halved from baseline to 6 months with denosumab (0.23 [0.18 to 0.33] to 0.11 [0.08 to 0.17]  $\mu\text{g/L}$ ) and alendronic acid (0.20 [0.14 to 0.28] to 0.09 [0.08 to 0.13]  $\mu\text{g/L}$ ) but were unchanged with placebo (0.23 [0.17 to 0.30] to 0.26 [0.16 to 0.31]  $\mu\text{g/L}$ ; Figure 2).

## Primary Endpoint

The primary endpoint was calculated in 136 participants (46 placebo, 46 denosumab, 44 alendronic acid; Figure 1) with a median time to final scan of 784 [770 to 793] days, in whom the overall aortic valve calcium score at baseline was 1110 [622 to 1998] AU. Compared to placebo, there were no differences in the 24-month change in aortic valve calcium score for either denosumab or alendronic acid (denosumab 343 [198 to 804] AU *versus* placebo 354 [76 to 675] AU,  $p=0.41$ ; alendronic acid 326 [138 to 813] AU *versus* placebo 354 [76 to 675] AU,  $p=0.49$ ; Figure 3A; Table I in the Supplement).

Mixed-effects linear regression showed no evidence of a difference between trial arms for either the denosumab-placebo model (least squares mean 1768 (95% CI 1434 to 2101) AU *versus* 1599 (95% CI 1262 to 1936) AU, difference in means 169 (95% CI -304 to 643) AU,  $p=0.48$ ) or the alendronic acid-placebo model (least squares mean 1792 (95% CI 1452 to 2132) AU *versus* 1596 (95% CI 1253 to 1939) AU, difference in means 196 (95% CI -286 to 679) AU,  $p=0.42$ ). Pre-specified sensitivity analyses limited to those at least 50% ( $n=129$ ) or 70% ( $n=118$ ) compliant demonstrated no differences in the primary outcome (Table II in the Supplement). A further sensitivity analysis of the primary outcome excluding calcium scores affected by artefact in 19 participants also demonstrated no differences in the primary outcome.

## Secondary Endpoints

Twenty-four month change in peak aortic jet velocity was calculated in 136 patients (46 placebo, 46 denosumab, 44 alendronic acid) with a median time to final echocardiogram of 780 [749 to 798] days, in whom the baseline peak aortic jet velocity was 3.35 [2.91 to 3.77] m/s. There were no differences in the calculated 24-month change in peak aortic jet velocity, either between denosumab and placebo (0.49 [0.15 to 0.75] *versus* 0.33 [0.12 to 0.59] m/s,  $p=0.21$ ) or between

alendronic acid and placebo (0.44 [0.11 to 0.63] *versus* 0.33 [0.12 to 0.59] m/s,  $p=0.74$ ; Figure 3B). There were no statistically significant between-group differences in calculated 24-month change in mean gradient or aortic valve area (Table III in the Supplement).

Aortic valve  $^{18}\text{F}$ -NaF uptake was measured in 130 participants (46 placebo, 44 alendronic acid, 40 denosumab) who underwent baseline and 12-month PET-CT (median time to scan 418 [406 to 429] days). There were no differences in the calculated 12-month change in aortic valve  $\text{TBR}_{\text{mean}}$  either between denosumab and placebo (0.00 [-0.11 to 0.16] *versus* 0.03 [-0.19 to 0.15],  $p=0.87$ ) or alendronic acid and placebo (0.06 [-0.09 to 0.21] *versus* 0.03 [-0.19 to 0.15],  $p=0.20$ ; Figure 3C). There were no differences in the 12-month change in aortic valve  $\text{TBR}_{\text{max}}$  either between denosumab and placebo (-0.02 [-0.19 to 0.40] *versus* 0.01 [-0.29 to 0.31],  $p=0.61$ ) or alendronic acid and placebo (0.12 [-0.12 to 0.40] *versus* 0.01 [-0.29 to 0.31],  $p=0.15$ ). There were no differences in calculated 12-month change in  $\text{SUV}_{\text{mean}}$  or  $\text{SUV}_{\text{max}}$  between groups (Table I in the Supplement). Baseline aortic valve  $^{18}\text{F}$ -NaF  $\text{TBR}_{\text{mean}}$  and  $\text{TBR}_{\text{max}}$  correlated with the calculated 24-month change in aortic valve calcium score ( $r=0.39$  and  $r=0.40$ ,  $p<0.001$  for both) and peak aortic jet velocity ( $r=0.26$  and  $r=0.25$ ,  $p=0.002$  and  $0.005$  respectively) (Figures I and II in the Supplement).

### Clinical and Safety Outcomes

A total of 41 participants (10 placebo, 14 denosumab, 17 alendronic acid) did not complete the final 24-month visit, 27 of whom attended at least one follow up visit and therefore contributed to the primary endpoint (Figure 1). There were 3 deaths in each of the study arms prior to the final study visit. There were no differences in the median number of adverse events (placebo 2 [1 to 3], alendronic acid 2 [1 to 2], denosumab 2 [1 to 3]) or serious adverse events (0 [0 to 1] for all groups) (Tables IV-VI in the Supplement). One serious adverse event was deemed related to a

study drug (alendronic acid): oesophagitis leading to dysphagia approximately 10 months after the study baseline visit, diagnosed on endoscopy and treated with proton pump inhibition and cessation of the study drug. No participants were unblinded during the study.

## Discussion

In this single-center parallel group double-blind randomized controlled trial, we demonstrate that treatment with denosumab or alendronic acid had no significant effect on the progression of aortic valve calcification over 24 months in asymptomatic patients with calcific aortic stenosis. We confirmed that both active trial interventions achieved inhibition of bone resorption but were unable to demonstrate an impact on the progression of aortic stenosis. We conclude that these treatments for osteoporosis do not have a major impact on the progression of aortic stenosis.

There remains a major unmet need for an effective disease-modifying non-invasive therapy in aortic stenosis. Following the failure of lipid-lowering therapies<sup>1-3</sup>, we hypothesised that targeting active valvular calcification might be a feasible therapeutic avenue to slow disease progression. This hypothesis was based on pre-clinical data demonstrating the importance of molecular triggers for calcification in the valve, as well as clinical observational data showing the close association between calcification activity measured with <sup>18</sup>F-NaF PET and the subsequent progression of aortic valve calcification and stenosis severity<sup>12, 13, 22, 32</sup>. Moreover, there have been several reports linking increased bone resorption and osteoporosis with calcification in the aorta and aortic valve<sup>33, 34</sup>, and the role of active aortic valve calcification has been highlighted in previous consensus statements<sup>35</sup>. The close associations between osteoporosis, bone turnover and calcific aortic stenosis led to our repurposing of drugs used to treat osteoporosis to test this hypothesis. Our results did not reject the null hypothesis.

Given the failure to meet the primary endpoint, it is important to consider the potential reasons for this. First, was the trial intervention successfully applied and did it achieve the desired pharmacological effect? Compliance was excellent in all study arms and there were no differences between the active arms and those receiving placebos. This finding confirms that the active interventions were well tolerated in this population of patients with aortic stenosis. Furthermore, we observed the expected halving of serum C-terminal telopeptide concentrations in those receiving denosumab or alendronic acid, confirming the pharmacodynamic effect of these drugs on bone turnover and resorption in our study population. We can therefore be confident that the trial interventions were successfully administered and achieved their anticipated pharmacological effects.

We should consider whether we have failed to detect an effect of the intervention because of insensitivity of the measurements of aortic stenosis progression or a lack of power. We set out to undertake a comprehensive assessment of aortic stenosis severity and progression using three complementary but distinctly independent methods: aortic valve calcium scoring, Doppler echocardiography and <sup>18</sup>F-NaF PET-CT. Aortic valve calcium scoring and echocardiography are standard clinical tools used to assess disease severity<sup>24, 36, 37</sup>, and we were able to identify and to quantify disease progression across all three trial treatment arms using both of these methods. We observed an overall rate of hemodynamic progression that is consistent with published series and trials<sup>1-3, 38</sup>. In addition, we also demonstrate that baseline <sup>18</sup>F-NaF PET, a measure of calcification activity, correlated with progression of both peak aortic jet velocity and aortic valve calcium score. This confirms our prior observational data demonstrating similar correlations<sup>22</sup>. Thus, these techniques have assessed drug efficacy from three distinct but



complementary approaches and found a concordant lack of effect in the two active trial interventions.

We acknowledge that our sample size was modest, with a preponderance of males. In addition, a proportion (13%) of patients developed a clinical indication for aortic valve replacement and did not complete the full 24-month study period. Importantly, many of these patients still contributed to the study endpoints, based on the available imaging data at their final visit. The proportion of patients who did not complete the full study period is consistent with the severity, profile and completion rates of previously reported randomized controlled trials of aortic stenosis therapies<sup>1-3</sup>. These factors were anticipated and accounted for in our sample size calculations and statistical analysis plan. Our trial population recapitulated the same rates of disease progression, including the anticipated increase in aortic valve calcium score which was our pre-specified primary endpoint. We found no signal towards benefit or harm in either active treatment arm, and the 95% confidence intervals encompassed our pre-specified effect size of 40%.

Would a larger study in a population with less severe aortic stenosis demonstrate a difference? The vast majority (85%) of our trial population had mild or moderate disease. The natural history of aortic stenosis dictates that many years will elapse before mild aortic stenosis will become severe. As the trial was powered for an effect size of 40%, it is possible we failed to detect a smaller treatment effect that could delay surgery in the longer term. However, we have recently examined the contemporary use of aortic valve calcium scoring for assessing disease progression and demonstrated that modest sample sizes, not dissimilar to our present study, are needed to detect the desired effect size sought here<sup>24</sup>.

Given that the trial intervention achieved its intended pharmacological effect and that multiple measures of disease severity and progression did not detect a treatment effect, was the underlying hypothesis incorrect? The molecular mechanisms underlying our hypothesis have been demonstrated in pre-clinical models, but the direct *in vivo* exploration of human valvular interstitial cell osteoclastic and osteoblastic differentiation and turnover has not been established and would be very challenging to undertake. We have previously demonstrated that microcalcification activity correlates well with aortic stenosis progression as measured by both aortic valve macrocalcification on non-contrast CT and hemodynamic stenosis on Doppler echocardiography<sup>22</sup>. Severe aortic valve calcification on non-contrast CT is also strongly associated with future aortic valve replacement<sup>31</sup>. We therefore continue to believe that valvular calcification remains a major pathogenetic determinant of aortic stenosis progression. However, there are multiple other pathways which lead to calcification in the valve, with many pro-inflammatory mediators that initiate and promulgate disease progression. We chose denosumab and alendronic acid as established treatments for osteoporosis in an attempt to slow valvular calcification whilst maintaining bone health. The absence of a detectable beneficial effect on valvular calcification may suggest that the pathophysiology of calcification in aortic stenosis is independent of these pathways, or that much higher doses than those used for the treatment of osteoporosis may have been needed. However, given the observed effect on markers of bone turnover, we believe the latter explanation is unlikely.

The main strength of our study is its rigorous design and the use of multiple measures of disease severity and activity to assess drug efficacy. It is the first double blind randomized controlled trial to test the hypothesis in question and has provided a clear answer, with concordant results across each imaging modality. However, the study is limited by its single

center design and a population skewed in ethnicity that, although representative of Scotland, may not be more widely generalizable. The under-representation of females in this study is an issue that we and others have encountered in previous similar trials and is clearly suboptimal<sup>1-3</sup>. We did include a small number (n=11, 7%) of patients with bicuspid aortic valves. This may be a potential confounder, as the calcific and non-calcific mechanisms underlying valve degeneration may differ from tricuspid aortic valves. We would also highlight that this trial was powered to investigate disease progression rather than clinical events. However, elective aortic valve replacement is largely based on symptom assessment and non-invasive measurements of aortic stenosis severity, and we have clearly demonstrated no treatment effect on the latter. Given the long time-course of aortic stenosis, it would not have been feasible to demonstrate a difference in clinical events during the relatively short 24-month study period. However, previous trials have demonstrated concordance between measures of disease severity and subsequent large-scale clinical endpoint trials in aortic stenosis<sup>1-3</sup>.

In conclusion, we have demonstrated that denosumab and alendronic acid have no effect on the progression of aortic valve calcification or stenosis severity over 24 months in patients with asymptomatic aortic stenosis. Alternative pathways and mechanisms need to be explored in order to identify a disease-modifying therapy for this growing population of patients with a potentially fatal condition.

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### **Disclosures**

None.

### **Supplemental Materials**

Supplementary Tables I-VI

## Supplementary Figures I-II

Trial Steering Committee members

Trial protocol, version 11, 29<sup>th</sup> Oct 2019

Protocol version tracker

Trial statistical analysis plan, version 3.2, 17<sup>th</sup> April 2020

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**Table 1.** Baseline Characteristics

Characteristic	Overall n = 150	Placebo n = 50	Denosumab n = 49	Alendronate n = 51
<b>Clinical</b>				
Age (years)	73±8	72±7	72±8	73±8
Female	31 (21%)	10 (20%)	11 (22%)	10 (20%)
White Scottish	136 (91%)	46 (92%)	44 (90%)	46 (90%)
Hypertension	114 (76%)	41 (82%)	35 (71%)	38 (75%)
Hypercholesterolemia	91 (61%)	35 (70%)	34 (69%)	22 (43%)
Type 2 diabetes mellitus	35 (23%)	12 (24%)	12 (24%)	11 (22%)
Chronic kidney disease	12 (8.0%)	2 (4.0%)	6 (12%)	4 (7.8%)
Chronic liver disease	1 (0.7%)	1 (2.0%)	0 (0%)	0 (0%)
Osteoporosis	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Prior angina	39 (26%)	13 (26%)	12 (24%)	14 (27%)
Previous myocardial infarction	17 (11%)	4 (8.0%)	8 (16%)	5 (9.8%)
Previous PCI	33 (22%)	11 (22%)	9 (18%)	13 (25%)
Previous CABG	15 (10%)	8 (16%)	3 (6.1%)	4 (7.8%)
Previous TIA/CVA	20 (13%)	6 (12%)	9 (18%)	5 (9.8%)
Malignancy	31 (21%)	10 (20%)	8 (16%)	13 (25%)
Current smoker	13 (8.7%)	3 (6.0%)	2 (4.1%)	8 (16%)
Ex-smoker	77 (51%)	27 (54%)	26 (53%)	24 (47%)
Height (m)	1.71±0.09	1.70±0.08	1.71±0.10	1.71±0.08
Weight (kg)	86 [76 to 93]	85 [79 to 91]	85 [76 to 91]	88 [76 to 100]
Systolic blood pressure (mmHg)	150±19	150±19	149±20	150±20
Diastolic blood pressure (mmHg)	78±11	77±10	79±12	76±11
Heart rate (/min)	67 [59 to 77]	70 [62 to 76]	67 [59 to 80]	66 [56 to 75]
C-terminal telopeptide (µg/L)	0.22 [0.16 to 0.30]	0.22 [0.17 to 0.30]	0.23 [0.18 to 0.32]	0.20 [0.14 to 0.27]
<b>Imaging</b>				
Bicuspid valve	11 (7.3%)	5 (10%)	3 (6.1%)	3 (5.9%)
Peak aortic jet velocity (m/s)	3.36 [2.93 to 3.82]	3.27 [3.03 to 3.73]	3.40 [2.89 to 3.80]	3.38 [2.86 to 3.87]
Mean aortic valve gradient (mmHg)	23 [18 to 32]	22 [18 to 29]	24 [18 to 33]	24 [18 to 32]
Stroke volume index (mL/m <sup>2</sup> )	42 [37 to 47]	42 [38 to 47]	41 [35 to 49]	42 [36 to 49]
Aortic valve calcium score (AU)	1,152 [655 to 2,065]	1,127 [617 to 2,059]	1,163 [598 to 2,151]	1,268 [672 to 2,065]
Aortic valve 18F-NaF TBRmax	2.57 [2.21 to 3.07]	2.49 [2.22 to 3.00]	2.79 [2.34 to 3.14]	2.55 [2.10 to 3.17]
Aortic valve 18F-NaF TBRmean	1.67 [1.45 to 1.86]	1.56 [1.43 to 1.80]	1.72 [1.54 to 1.86]	1.66 [1.44 to 1.89]

Median [interquartile range]; mean±standard deviation; n (%)

Abbreviations: PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft surgery; TIA, transient ischemic attack; CVA, cerebrovascular accident; NaF, sodium fluoride; TBR, tissue to background ratio

## Figure Legends

**Figure 1.** CONSORT diagram. The primary endpoint (24-month change in aortic valve calcium score) was calculated from a daily rate of change based on the difference between baseline and final aortic valve calcium score, whether this was at 12 months or 24 months.

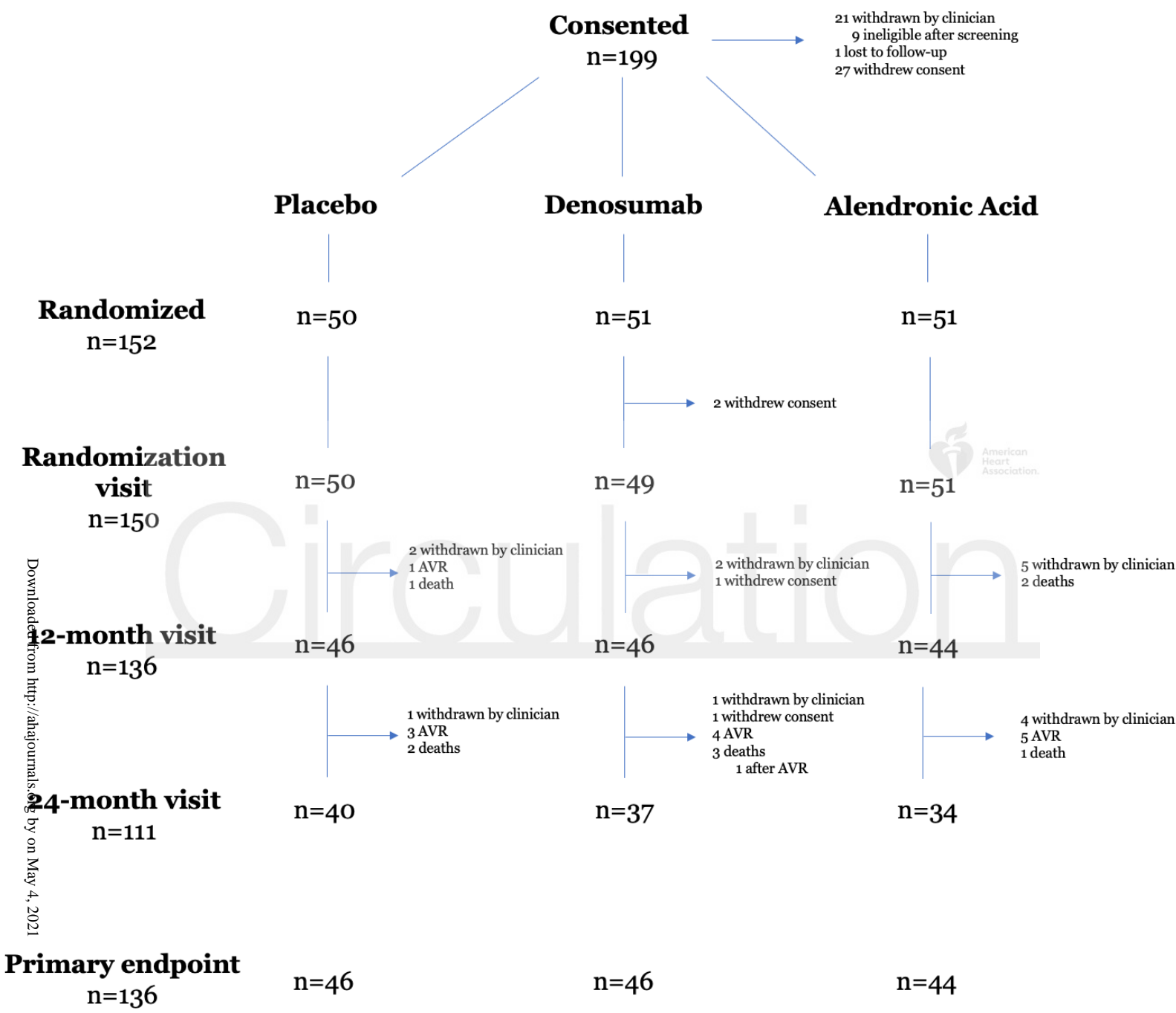
Abbreviations: AVR, aortic valve replacement.

**Figure 2.** A: C-terminal telopeptide concentrations at baseline and 6 months for each trial arm (p values: placebo >0.5, denosumab <0.001, alendronic acid <0.001; Wilcoxon rank sum test).

B: Six-month change in C-terminal telopeptide for each trial arm

(p<0.001 for both denosumab and alendronic acid compared to placebo; Wilcoxon rank sum test).

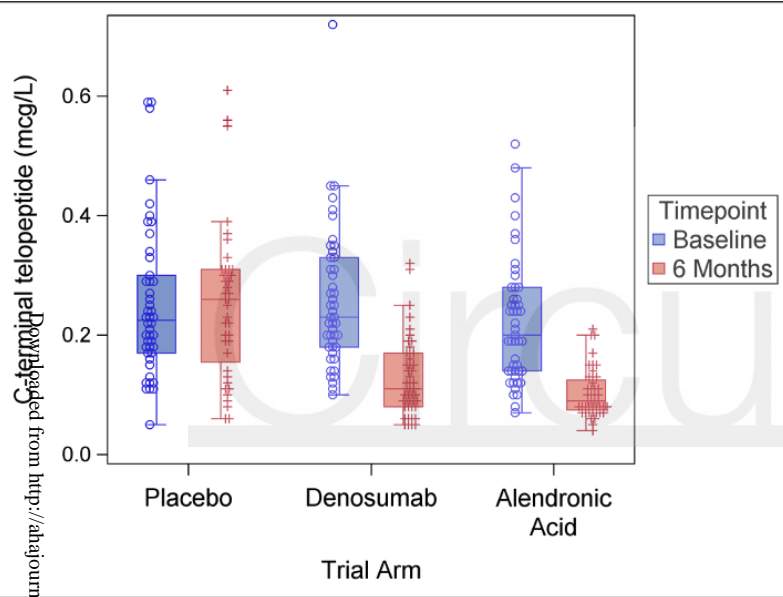
**Figure 3.** Primary and key secondary endpoints. (A) Calculated change in 24-month aortic valve calcium score (p=0.41 for denosumab vs placebo; p=0.49 for alendronic acid vs placebo; Wilcoxon rank sum test), (B) calculated change in 24-month peak aortic jet velocity (p=0.21 for denosumab vs placebo; p=0.74 for alendronic acid vs placebo; Wilcoxon rank sum test), and (C) calculated change in 12-month aortic valve maximum target to background ratio (p=0.61 for denosumab vs placebo; p=0.15 for alendronic acid vs placebo; Wilcoxon rank sum test).



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**A**



**B**

