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Denosumab as a pharmacological countermeasure against osteopaenia in long duration spaceflight

--Manuscript Draft--

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06-Jan-22

Editorial Committee
Aerospace Medicine & Human Performance

Dear Editor(s),

We invite you to review our paper, titled "Denosumab as a potential pharmacology countermeasure against microgravity-induced osteopaenia: A review and a proposal", for consideration for publication in your journal Aerospace Medicine & Human Performance. This paper has been part of a project that was undertaken independently by the authors to evaluate the literature surrounding the use of pharmacological agents to prevent the loss of bone density in spaceflight. The idea stemmed from my own professional experience in using denosumab in patients with post-menopausal osteoporosis, noticing not only improved compliance and reduced side-effects compared to traditional treatments, but that the literature overall supported superior outcomes.

Whilst undertaking our review of the literature, both Vienna and I have presented our summary and proposal at numerous scientific meetings during 2021, including COSPAR21, the International Society of Gravitational Physiology and at the Australasian Society of Aerospace Medicine's annual meeting. During this time, we also invited Dr Li Sheen Toh, a clinical pharmacologist with an interest in space pharmacology, to assist with our paper.

Our review provides a good overview of the current state of countermeasures used in spaceflight for bone health, as well as providing solid discussion around future directions of research in pharmaceuticals (not purely limited to denosumab). Considering the readership of AMHP is extensive, particularly amongst the space medicine community, we have submitted to your journal as our first choice for publication.

I hope that you and the editorial committee enjoy our article and find it informative. We look forward to your commentary eagerly.

Yours Truly,

Recoverable Signature

X 

Signed by: 5cb69cb9-bf76-4934-a4ac-5c16ec960f6b

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07-Jan-23

Editorial Committee
Aerospace Medicine & Human Performance

Dear Editors and Referees,

We thank you for taking the time to for review our paper for submission, AMHP6053R4, titled "Denosumab as a pharmacological countermeasure against osteopaenia in long duration spaceflight".

Following your recent feedback and requests for revisions, the paper has been further reviewed and edited by the author group. In this revised manuscript, the following changes have occurred:

- At the suggestions of the 1st reviewer, the speculative comments on hormonal effects of osteoporosis have been removed.
- The introduction has been significantly shortened and condensed. Discussion of pharmacological measures begins in the second paragraph, as opposed to the fourth paragraph in the previous revision.
- De-emphasized mission to Mars and instead referenced long-duration spaceflight to be more encompassing of all planned future exploration missions.
- The section on normal bone physiology, exercise and nutritional countermeasures have been removed.
- The section on alendronate has been shortened.
- A PRISMA-style diagram regarding the article selection has been added
- A table summarizing the pros and cons of alendronate and Densoumab has been added
- At the 2nd reviewer's suggestion, a greater variety of adverbs and conjunctions have been used as opposed to whilst.
- Double spacing has been introduced throughout.
- Affiliations have been added and updated on the title page
- Running title in top corner removed – with only page numbers present in the header
- Reference list has been checked manually. Minor errors corrected. All DOI/URL links are correct.
- The acknowledgement of Mrs Grace Mowtschan in her role with proofing and editing has been confirmed and emailed to the editors.

Finally, the second reviewer had suggested that the proposed studies be removed. As our literature review was conceived to encourage further research in pharmaceuticals as countermeasures for spaceflight, we as the authors believe it was integral to our discussion to include an overview of a potential research protocol. However, acknowledging the reviewers concern and that this manuscript is a literature review as opposed to a formal research proposal, we have significantly reduced the discussion on the proposed studies. What remains will allow for further conversion and conjecture on the topic and highlights the authors views on future directions in this area.

These further revisions have resulted in a smaller and more focussed paper that will now hopefully meet the journals standards and the satisfaction of the reviewers and editorial staff.

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Yours Sincerely,

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Title:

Denosumab as a pharmacological countermeasure against osteopaenia in long duration space flight

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Abstract

Introduction

Prolonged exposure to microgravity is associated with a significant reduction in bone density, exposing astronauts to renal calculi in flight and osteoporotic fractures on return to Earth. Whilst physical countermeasures and bisphosphonates may reduce demineralization, additional therapies are needed for future interplanetary missions. This literature review aims to understand the current background pertaining to denosumab, a monoclonal antibody therapy used in osteoporosis, and its potential use for long duration spaceflight.

Method

A literature review was conducted using the keywords “osteoporosis”, “osteopaenia”, “microgravity”, “space flight”, “bed rest”, “denosumab”, “alendronate”, “bisphosphonates” and “countermeasures”. Additional articles were identified through references. Forty eight articles, including systematic reviews, clinical trials, practice guidelines and textbooks were included for discussion.

Results

No previous bed rest or in-flight studies regarding denosumab were identified. In osteoporosis, denosumab is superior to alendronate in maintaining bone density with a lower rate of side-effects. Emerging evidence in reduced biomechanical loading state suggests denosumab improves bone density and decreases fracture risk. Concerns exists over vertebral fracture risk following discontinuation. The dosing regimen of denosumab offers practical advantages over bisphosphonates. Existing spaceflight studies with alendronate serve as a template for a study with denosumab and allow for a direct comparison of efficacy and safety.

Discussion

Denosumab has numerous potential advantages as a countermeasure to microgravity-induced osteopaenia when compared to alendronate, including improved efficacy, less side-effects, better tolerability and convenient dosing regimen. Two further studies are proposed to determine in-flight efficacy and the suitability of monoclonal antibody therapy in the spaceflight environment.

Key Words

denosumab; osteopaenia; osteoporosis; microgravity; spaceflight; bone density; alendronate; bisphosphonate

Introduction

With humanity entering the next generation of space exploration, astronauts will be exposed to prolonged periods of microgravity and confinement beyond that of current missions in low earth orbit. On the International Space Station (ISS), astronauts experience a decrease in bone mineral density (BMD) at a rate of 1-2% month, with the greatest loss in the lower limbs[9, 38, 45]. This exposes astronauts to a significant increase in the risk of fractures on return to Earth, as well as renal calculi and cardiovascular events in-flight[11, 32, 47]. The National Space & Aeronautics Administration (NASA) has identified in the Human Research Roadmap that bone demineralisation is a serious threat to astronaut health and therefore has prioritised the research into the development of countermeasures[33].

Specific exercise countermeasures including the Advanced Resistive Exercise Device (ARED)[39], combined with load bearing garments and nutritional regimes[40], have been extensively assessed on the ISS. Despite showing a maintenance of BMD in the upper body, significant loss still occurs in the lumbar spine and femur[38-40]. Although exercise may be considered sufficient for short duration spaceflights, pharmacological countermeasures should be considered for longer spaceflights. To date, only alendronate has been assessed with a bed-rest study[26] and clinical trial during spaceflight[25]. Whilst demonstrating maintenance of bone density, the method of administration and common side effects are an issue, with two out of eleven astronauts participating in the inflight study withdrawing due to gastrointestinal side effects[25].

Numerous advancements in osteoporosis pharmacotherapy have occurred in the last decade, including the introduction of the monoclonal antibody denosumab. Acting as an inhibitor of the receptor activator of nuclear factor kappa-B (RANK) ligand (RANKL), denosumab selectively inhibits osteoclast activity via the RANKL-Osteoprotegerin (OPG) axis without impeding osteoblast activity to maintain bone density[1]. Unlike other anti-resorptive therapies, it is available in a convenient 60mg subcutaneous injection delivered six monthly[1, 27]. Furthermore, systematic reviews comparing denosumab to bisphosphonates in osteoporosis demonstrated better rates of compliance, as well as improved long-term

outcomes in maintenance of bone density[2, 8, 24]. Despite its real-world efficacy, it is not mentioned in the Human Research Roadmap as a potential pharmacological countermeasure for long-duration spaceflight. However, the recent European Space Agency SciSpace white papers on pharmacological countermeasures highlighted the need for a strategy to review new terrestrial therapies for space application[7].

The aim of this literature review is to understand the current literature pertaining to denosumab use and potential in long duration spaceflight. This paper summarises current knowledge of pharmacological countermeasures pertaining to bone health in spaceflights, discussing the potential benefits and disadvantages of both denosumab and alendronate. It then proposes further studies to assess the stability of monoclonal antibody therapies in spaceflight and efficacy of denosumab as a countermeasure against microgravity-induced osteopaenia.

Methods

To assess the current knowledge of osteopaenia in microgravity and its management, as well as the current evidence regarding the use of alendronate and denosumab, a literature review was undertaken. A search was conducted through the University of Otago library, (which includes Medline, EMBASE, Scopus and Pubmed databases), using the terms “osteopaenia”, “osteoporosis”, “bone”, “skeletal”, “bed rest”, “microgravity”, “countermeasures”, “space”, “alendronate”, “bisphosphonate” or “denosumab”. These terms were selected to broadly identify papers of interest, with additional articles identified through references of found literature, clinical practice guidelines, textbooks and from material provided by drug manufacturers.

Though no strict inclusion or exclusion criteria were used, clinical studies from prior to 1990 were excluded due lack relevance in contemporary practice in osteoporosis as well as current space research. In addition, studies investigating anti-resorptive therapies in malignancy were generally excluded. As the focus of the review primarily concerns denosumab and alendronate, articles detailing other anti-resorptive agents and/or nutritional

supplementation were excluded. However, one new monoclonal antibody therapy, romosozumab, which has been trialled with denosumab, was identified and thus included for discussion. While no form of meta-analysis was undertaken, relevant statistics derived from clinical research are quoted in this paper.

Therefore, this literature review draws from a total of forty eight articles including systematic reviews, meta-analyses, randomized control trials, case studies, product information, textbooks and clinical guidelines. A summary of the selection process is seen in Figure I.

Results

Changes During Space Flight

Cumulative data from across the Apollo, Space Shuttle, Mir and ISS missions shows that a prolonged exposure to microgravity results in bone resorption and a total BMD loss rate of 1-2% per month[9, 38, 45]. However, the greatest loss is seen in the high load bearing bones, with up to 20% loss in the femur, pelvis and lumbar spine[45, 47]. The loss in BMD is persistent after spaceflight, with only 50% of pre-flight BMD in the trochanter recovered by 9 months and returning to baseline by 3 years[38].

In addition to the five-fold increased risk of fractures, the rapid resorption of bone during early spaceflight may result in hypercalcaemia and hypercalciuria, contributing to the formation of renal calculi and atherosclerotic disease[11, 41, 47]. In comparison, terrestrial bone loss in older populations at the femoral neck is estimated at 0.82-0.96% per year[23]. It is postulated that a lack of activity in anti-gravity extensor muscles in the lower limb and trunk reduces tension on the surrounding bones, which, when combined with the lack of mechanical force normally induced by gravity, results in rapid reductions in bone density[11, 32, 47].

Currently, astronauts on the ISS perform aerobic and resistive exercise for up to 2.5 hours daily, inclusive of setup time[11]. When combined with strict adherence to the assigned diet with sufficient calorie, calcium and vitamin D intake, overall BMD is maintained in the upper body[40]. However, significant loss is still seen in the lumbar spine, femur and pelvis;

associated with elevation of resorption markers C-terminal telopeptide (CTX) and N-terminal telopeptide (NTX)[39, 40]. Whether the BMD decrease observed was associated with clinically significant bone geometry changes, including cortical thinning at the femoral neck, is not known due to CT imaging not being performed in either study.

Regardless of physical activity in-flight, increased levels of CTX and NTX are observed compared to pre-flight measurements[42, 43], indicating increased osteoclast activity. Due to bone resorption, there is an increased risk of renal stone formations from increase urinary calcium and oxalate excretion[40, 42, 43]. Whilst baseline differences in bone biochemistry may exist between sexes prior to spaceflight, there is no difference in response to microgravity in the maintenance of bone density[43].

Therefore, even with current countermeasures, astronauts on prolonged spaceflights are at risk of osteoporotic fractures in loadbearing bones upon return to a normal gravitational environment, as well as at elevated risk of renal calculus formation. However, numerous biochemical mechanisms that exist through which bone density can be altered or maintained in microgravity - through altering calcium homeostasis, enhancing bone deposition, or via selectively targeting the RANKL-OPG axis.

Alendronate

Bisphosphonates are structurally similar moieties to pyrophosphate, with a high affinity for calcium and thus accumulate within skeletal tissue. Nitrogenous bisphosphonates, including alendronate and zoledronate, are antagonists of farnesyl diphosphate synthase, which interrupts sub-surface protein trafficking in osteoclasts. This alters the cytoskeletal structure required for bone contact and, hence, inhibits osteoclastogenesis and bone resorption[27].

Alendronate is taken as an oral tablet, either daily, weekly or monthly[35]. It can be stored for prolonged periods in a well-sealed container between 15-30°C[35]. As dietary calcium, magnesium and aluminium interact with bisphosphonates, alendronate must be taken when fasted and at least 30 min prior to food. It must be taken sitting upright to minimize gastroesophageal reflux – a common side-effect, which often contributes to reduced

compliance and discontinuation. This may be troublesome in microgravity, as astronauts are unable to sit upright and gastric emptying is often delayed during early spaceflight due to space motion sickness[22]. An alternative, zoledronate is given as a yearly IV infusion, which avoids the majority of gastrointestinal side-effects though post-infusion arthralgias are very common[34].

Bisphosphonates are also associated with two rare phenomena. Osteonecrosis of the jaw can occur spontaneously; however, there is associated with dental trauma, poor oral health, and as a function of dose and number of years on therapy[36]. Atypical femur fractures are atraumatic, occurring along the diaphysis (shaft or subtrochanteric) and, like stress fractures, are transverse[15, 27]. Counterintuitively, the atypical fracture risk increases with long-term therapy, with annual incidence of 1.78 per 100,000 with <1.9 years use increasing to 113.1 per 100,000 for >8 years use[15]. Both conditions are considered unlikely to occur in the astronaut population, due to their high baseline of health and that therapy duration is unlikely to extend much beyond the three years of an expected return Mars mission.

To date, alendronate is the only pharmacological agent to be assessed as a countermeasure against microgravity-induced osteopaenia[25]. A 17-week bed rest study demonstrated that, compared to controls, administration of alendronate not only maintained bone density in all bones examined (with exception of the calcaneus) but also suppressed markers of bone turnover and loss of calcium[26]. A follow-up inflight study examined ten astronauts who were prescribed 70mg alendronate weekly, commencing three weeks prior to a 5.5 month ISS expedition and continuing throughout[25]. Astronauts were required to undertake the normal 2.5 hour daily exercise regime during the study.

BMD at the femoral neck, trochanter, total hip, pelvis and lumbar spine was assessed pre- and post-flight using dual-energy X-ray absorption (DXA) and quantitative CT (QCT). Markers of bone turnover, including urinary and serum calcium, NTX, CTX, Vitamin D and PTH, were recorded at specified intervals pre, during and post flight. The data was compared to historical ISS data of eighteen astronauts who undertook ISS missions prior to 2008 using the interim resistive exercise device and missions post 2008 with eleven

astronauts who used the ARED. Of note, no QCT was available for the ARED group for comparison to the alendronate group. All astronauts continued to take standard vitamin D and calcium supplementation with a normal expedition diet. During the study, three astronauts withdrew from the alendronate group – one for personal reasons, one from gastrointestinal discomfort following a test dose and one from developing dyspepsia in-flight[25].

Compared to the exercise only groups, the alendronate group showed a clinically significant maintenance of pre-flight bone density scores on DXA with relative suppression of markers of bone turnover[25]. There was also a significant difference in BMD and bone mineral content (BMC) on QCT across all sites between pre-ARED and the alendronate group[25]. As it was unclear as to whether the use of ARED in the alendronate group accounted for the significant difference in QCT BMD/BMC, a follow-up study recruiting an additional 10 astronauts using the same protocol and investigations was undertaken[39]. Although this demonstrated that ARED did ameliorate overall bone loss, it did not significantly reduce trabecular BMD and BMC loss in the hip nor suppress markers of bone turnover[39]. Therefore, it can be concluded that the effects in BMD maintenance seen is due to the anti-resorptive effect of alendronate[25, 39].

Denosumab

Denosumab is a novel human monoclonal antibody approved by the FDA in 2010 for treatment of osteoporosis and fracture prevention in bone metastasis, which acts a specific inhibitor of RANKL[1, 27]. RANK is expressed on the surface of pre-osteoclasts and by binding to RANKL, triggers maturation into osteoclasts. The maturation of osteoclasts is controlled by OPG, which is expressed by osteoblasts and regulated by exposure to oestradiol. It is thought that in osteoporosis that decreased oestradiol exposure leads to decreased OPG expression and hence unchecked osteoclast maturity[27]. This imbalance in osteoclast activity progressively leads to decreased bone density. Denosumab therefore inhibits RANKL to prevent osteoclast maturity in a manner akin to OPG but independent of oestradiol.

Denosumab is packaged in pre-drawn syringes, which must be protected from light, freezing and excessive vibration, being stored at 2-8°C until use[1]. Once removed from refrigeration, it may be kept in room temperature <25°C for up to 30 days[1]. For osteoporosis, it is administered as a 60mg subcutaneous injection every 6 months. Following administration, CTX falls rapidly and stabilize after three days[1]. While the peak serum concentration is reached in 10 days and the half-life is 26 days, denosumab has been shown to control bone resorption for up to 6 months, corresponding with suppressed CTX and maintenance of BMD during this period[1].

During the literature search, no previous bed rest or in-flight studies involving denosumab were identified. However, denosumab is currently recommended as an alternative first-line option to bisphosphonates for fracture prevention in osteoporosis due to its efficacy[1, 27, 46]. The FREEDOM blinded randomized control trial demonstrated significant reductions in relative risk of vertebral (68%), hip (40%) and non-vertebral (20%) fractures compared to placebo over three years of treatment[14]. In addition, this was accompanied by a 9.2% and 6.0% relative increase in total vertebral and hip BMD compared to placebo[14]. In the phase III DECIDE double blind randomized non-inferiority trial, the denosumab group showed a further 0.9% and 1.1% absolute increase in BMD measured via DXA compared to treatment alendronate at the hip and lumbar spine respectively[8]. The FREEDOM trial extension demonstrated that BMD continued to improve up to ten years, with up to 21.7% increase in lumbar spine and 9.2% in total hip from study baseline[6].

Furthermore, the efficacy of denosumab over alendronate has been confirmed in additional independent studies. A 2017 retrospective analysis assessed both agents over a 12-month period, with the denosumab group showing superior improvement in femoral neck density on DXA[24]. Though not seen in the alendronate group, denosumab showed significant increase in lumbar BMD[24]. A 2015 meta-analysis looking at multiple anti-resorptive therapies showed that denosumab was as efficacious as bisphosphonates at prevent hip fractures (denosumab OR 0.60, alendronate 0.61); however, it further demonstrated a significant

decrease in the risk of vertebral fractures (OR 1.67), echoing denosumab's real-world superiority and efficacy[48].

Pooled data from phase III trials[14] showed that the most commonly reported side-effects in denosumab and placebo groups were back pain (34.1% vs 34.0%), arthralgia (20.4% in both), hypertension (15.3% vs 16.1%), nasopharyngitis (14.8% vs 15.6%), pain in the extremities (11.8% vs 11.2%),osteoarthritis (10.9% vs 11.1%) , eczema (3.0% vs 1.7%) and skin infections (0.4% vs 0.1%)[1]. Pancreatitis was reported in 0.1% of cases versus 0.2% in the placebo group; however, most of these cases were due to pre-existing pathology including gallstones[1]. Of note, there was no significant statistical difference in rate of all and serious side-effects between treatment group placebo in the FREEDOM trial (p = 0.91 & 0.61)[14]. Anaphylaxis is rare with denosumab administration, with five reported cases in post-marketing surveillance and no fatal outcomes[20]. No evidence of neutralizing antibodies to denosumab have been reported[6, 14].

Hypocalcaemia is a potential concern with a dose-dependent effect. A head-to-head trial of 5677 patients with bone metastases, in which denosumab was administered at higher 120mg dose monthly, showed that hypocalcaemia occurred in 9.6% of denosumab patient compared to 5.0% of those treated with zoledronic acid[29]. Severe symptomatic hypocalcaemia requiring treatment with IV calcium occurred in 3.7% and 1.7% of cases in each respective treatment group[29]. No cases were seen within the denosumab group in the initial FREEDOM trial[14], with overall annual incidence remaining ≤0.1 per 100,000 in the ten year extension trial[6]. Post-marketing surveillance in 2013 of denosumab use in osteoporosis only identified eight cases of severe symptomatic hypocalcaemia, of which seven cases were associated with chronic kidney disease[20]. Though the risk of hypocalcaemia is low in the dose used in osteoporosis, it is recommended serum calcium is checked prior to commencement of denosumab and adequate dietary intake or supplementation with calcium and vitamin D is maintained during denosumab treatment[1, 17, 27].

While there was no reported incidences of osteonecrosis of the jaw or atypical femur fractures in the DECIDE trial[8], the FREEDOM extension trial recorded 14 cases of jaw

osteonecrosis and two of confirmed atypical femur fractures, with an overall annual incidence of ≤0.1 per 100,000 for both conditions respectively[6].

Discontinuation of denosumab in osteoporosis shows an elevation of resorption markers at 6 months, exceeding pre-treatment levels at 12 months, accompanied by a decline towards or below baseline BMD[30, 31]. Whilst these studies did not show an increased fracture risk in the groups that discontinued treatment[30, 31], a post-hoc analysis of the 10-year FREEDOM trial extension showed that there was an significant increase in single and multiple vertical fractures in the individuals who ceased denosumab[13], confirming the findings in a series of case reports[44]. However, it is noted that the rate does not exceed that of the placebo group and this was strongly associated with individuals who had a prior history of vertebral fractures[13]. Whilst recommencing denosumab results in clinically significant improvements in BMD after a period of discontinuation[6], treatment guidelines for osteoporosis warn against drug holidays or if being discontinued, that bridging therapy such as a bisphosphonate is considered[17, 27, 46].

This may be a potential concern for astronauts returning after a long-duration spaceflight, who may need to take an anti-resorptive agent for a period following a return to Earth. However, further data is needed in a younger and healthier populations to confirm whether the discontinuation effect occurs outside of the elderly populations previously studied. A summary of denosumab compared to alendronate is presented in Table I.

Future applications

Cirnigliaro et al 2020 investigated the use of denosumab in maintaining lower limb BMD in total motor spinal cord injury patients[10]. Even without exercise countermeasures, significant maintenance of bone density occurs when administered shortly after injury, with control groups showing >10% density loss and up to 43% increased absolute risk of lower limb fractures compared to the denosumab treatment group[10]. Whilst no bedrest studies have been performed on healthy neurologically intact individuals, this is the first study to show the role of denosumab in maintaining density in a low mechanical loading setting, which could

be considered analogous to the unloading seen in microgravity. Further bed rest studies could be used to confirm this finding in healthy individuals.

Other potential therapies

During the literature review, an additional agent that has been trialled with denosumab was identified. Romosozumab is a novel monoclonal antibody that targets sclerostin and which has recently been approved for treatment in post-menopausal osteoporosis[4]. Sclerostin is secreted by osteocytes and acts as an anti-anabolic agent via the Wnt signally pathway, inhibiting osteoblast activity[27]. Its secretion is regulated by mechanical loading as well as PTH and oestrogen[27].

A large international study assessing vertebral fracture risk randomized post-menopausal women to receiving either 210mg romosozumab monthly for 12 months or placebo, followed by 6 monthly denosumab for an additional year[12]. In the romosozumab group, vertebral fractures occurred in 0.6% of participants at 24 months compared to 2.5% placebo group[12]. However, there are no published trials comparing it as a single agent head-to-head with other antiresorptive therapies. Also, unlike denosumab, it is administered by subcutaneous injection monthly instead of six monthly. It is only effective for up to 12 months, with 18% of patients being shown to develop neutralizing antibodies, which requires switching to a different antiresorptive agent[4]. Of concern is the reported increase in cardiovascular events with romosozumab, with a 2.5-4.9% incidence in treatment groups[3, 28, 37]. Thus, it is contraindicated in individuals with previous ischaemic heart and cerebrovascular disease[3]. The elevated cardiovascular risk associated with romosozumab may preclude its utility in long-duration spaceflight.

Discussion

This literature review is the first to discuss the use of denosumab for human spaceflight and has attempted to compare it to alendronate – a previously trialled agent on the ISS. It has shown that denosumab has real-world advantages over alendronate in the management of

osteoporosis and appears that denosumab could be an ideal candidate for long-duration spaceflight. It demonstrates no increase in common side-effects versus placebo or alendronate, with a lower rate of rare side-effects. Importantly, it does not cause the gastrointestinal side-effects seen in alendronate. The initial dose can be administered a fortnight prior to flight and have full effect once in microgravity. Additional doses would need to be carried for every six months of mission length and can be easily administered via a subcutaneous injection without the complications of PO or IV administration of bisphosphonates.

A limitation of this review is that there are no published trials regarding denosumab in bed rest or spaceflight and hence all potential benefits are inferred from terrestrial studies. In addition, as there is a paucity of research in pharmacological countermeasures in space, it was not possible to undertake a systematic review or meta-analysis. Therefore, further research is required to validate the use of denosumab in space, either with in bed-rest analogue studies and in actual spaceflight. Though it is anticipated that the spaceflight environment alters the pharmacokinetics, pharmacodynamics and the active pharmaceutical content in medication[16, 18, 21], no monoclonal antibody therapies have been assessed to date. Denosumab and monoclonal antibodies also have more stringent storage requirements compared to alendronate which may impact the suitability for spaceflight. The authors would encourage future spaceflights to have a dedicated storage area for medications needing cooler storage.

To assess denosumab's efficacy in long-duration spaceflight, the authors propose a study. Following on from LeBlanc et al 2013[25] and Sibonga et al 2019[39], a minimum of 10 astronauts will be recruited prior to a 3–6-month ISS expedition. We expect the study will show that the denosumab treatment group will demonstrate significantly greater maintenance of BMD compared to exercise (with or without GLCS) groups in the previous studies.

Whilst on the ISS, astronauts will continue to undertake a standard 2.5 hours per day exercise routine using the cycle ergometer and ARED[11]. Following the return to Earth,

biochemistry and BMD will be reassessed. This data will then be compared to historical study data regarding mechanical countermeasures and alendronate.

Due to potential discontinuation effects following cessation of denosumab, astronauts will need to be studied at 6- and 12-months post-flight to determine whether there is a rebound loss of bone density and increased risk of fractures. Consideration could be given to also studying whether a bridging agent such as alendronate could be used to ameliorate such a discontinuation effect, in line with clinical guideline recommendations[1, 17, 46]. The authors consider that in a healthy astronaut population the rebound effect may not be potentially seen, due the absence of the hormonal deficits and advanced ageing in the osteoporotic patients previously studied.

A limitation of this study would be that the ISS is situated within Earth's magnetosphere; therefore, not accurately reflecting the radiation environment seen while traversing the interplanetary medium[19]. An additional study could be conducted on Earth using a cyclotron with exposure to an appropriate radiation source. This would not only determine denosumab's viability and stability as a medication for an interplanetary mission but will serve as the first study examining pharmaceutical monoclonal antibodies in a spaceflight-like environment.

Finally, it is worthwhile discussing the role of maintaining a space pharmacopeia based on the best current practice on Earth. Before use in spaceflight, medication undergoes extensive and expensive bed rest studies before consideration of in-flight trials[5]. Not only does the cost input makes trialling new and emerging medication unattractive for a budget-strapped government agencies and private industry, but it also delays the introduction of potentially useful agents. Acknowledging the well-documented pharmacokinetic and pharmacodynamic changes in spaceflight, thought should be given to fast tracking the use of newer agents once there is a demonstrated safety record and efficacy on Earth-bound applications.

Conclusion

This literature review has examined denosumab as a potential countermeasure for microgravity-induced osteopaenia, shows that from its real-world performance, denosumab has numerous promising benefits that extend to long-duration spaceflight. Considering its superiority to alendronate, which has thus far been proven as a useful pharmacological countermeasure, denosumab should be considered for investigation at preventing microgravity-induced osteopaenia. The proposed studies may address the current knowledge gap surrounding the use of denosumab and monoclonal antibodies in spaceflight. As per the NASA Human Research Roadmap, by developing more robust countermeasure to prevent fractures and renal calculi, it will ensure the success of future astronauts undertaking interplanetary exploration.

Acknowledgements

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References

1. Amgen Australia. Prolia (Denosumab) Product Information 2022. Available from: http://www.guildlink.com.au/gc/ws/amgen/pi.cfm?product=anpprolm10315.

2. Anastasilakis AD, Toulis KA, Goulis DG, Polyzos SA, Delaroudis S, Giomisi A, et al. Efficacy and Safety of Denosumab in Postmenopausal Women with Osteopenia or Osteoporosis: A Systematic Review and a Meta-analysis. Horm Metab Res. 2009;41(10):721-9. https://doi.org/10.1055/s-0029-1224109.

3. Asadipooya K, Weinstock A. Cardiovascular Outcomes of Romosozumab and Protective Role of Alendronate. Arteriosclerosis, Thrombosis, and Vascular Biology. 2019;39(7):1343-50. https://doi.org/10.1161/ATVBAHA.119.312371.

4. Australian Prescriber. Romosozumab for osteoporosis. Australian Prescriber. 2021;44:109-10. https://doi.org/10.18773/austprescr.2021.021.

5. Blue RS, Bayuse TM, Daniels VR, Wotring VE, Suresh R, Mulcahy RA, et al. Supplying a pharmacy for NASA exploration spaceflight: challenges and current understanding. NPJ Microgravity. 2019;5:14. https://doi.org/10.1038/s41526-019-0075-2.

6. Bone HG, Wagman RB, Brandi ML, Brown JP, Chapurlat R, Cummings SR, et al. 10 years of denosumab treatment in postmenopausal women with osteoporosis: results from the phase 3 randomised FREEDOM trial and open-label extension. Lancet Diabetes Endocrinol. 2017;5(7):513-23. https://doi.org/10.1016/S2213-8587(17)30138-9.

7. Boutouyrie P, Derobertmasure A, Morbidelli L, Raghda R, Toh LS, Stingl J, et al. ESA SciSpacE White Paper #14: Pharmacological Countermeasures: European Space Agency; 2021. Available from: https://esamultimedia.esa.int/docs/HRE/14_HumanResearch_PharmacologicalCountermeasures.pdf.

8. Brown JP, Prince RL, Deal C, Recker RR, Kiel DP, de Gregorio LH, et al. Comparison of the effect of denosumab and alendronate on BMD and biochemical markers of bone turnover in postmenopausal women with low bone mass: A randomized, blinded,

phase 3 trial. Journal of Bone and Mineral Research. 2009;24(1):153-61. https://doi.org/10.1359/jbmr.0809010.

9. Carpenter RD, Leblanc AD, Evans H, Sibonga JD, Lang TF. Long-term changes in the density and structure of the human hip and spine after long-duration spaceflight. Acta Astronautica. 2010;67(1):71-81. https://doi.org/10.1016/j.actaastro.2010.01.022.

10. Cirnigliaro CM, La Fountaine MF, Parrott JS, Kirshblum SC, McKenna C, Sauer SJ, et al. Administration of Denosumab Preserves Bone Mineral Density at the Knee in Persons With Subacute Spinal Cord Injury: Findings From a Randomized Clinical Trial. JBMR plus. 2020;4(8):e10375. https://doi.org/10.1002/jbm4.10375.

11. Clément G. Fundamentals of Space Medicine. Second ed. New York, NY: Springer; 2011. doi: https://doi.org/10.1007/1-4020-3434-2.

12. Cosman F, Crittenden DB, Adachi JD, Binkley N, Czerwinski E, Ferrari S, et al. Romosozumab Treatment in Postmenopausal Women with Osteoporosis. New England Journal of Medicine. 2016;375(16):1532-43. https://doi.org/10.1056/NEJMoa1607948.

13. Cummings SR, Ferrari S, Eastell R, Gilchrist N, Jensen JB, McClung M, et al. Vertebral Fractures After Discontinuation of Denosumab: A Post Hoc Analysis of the Randomized Placebo-Controlled FREEDOM Trial and Its Extension. Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research. 2018;33(2):190-8. https://doi.org/10.1002/jbmr.3337.

14. Cummings SR, Martin JS, McClung MR, Siris ES, Eastell R, Reid IR, et al. Denosumab for Prevention of Fractures in Postmenopausal Women with Osteoporosis. New England Journal of Medicine. 2009;361(8):756-65. https://doi.org/10.1056/NEJMoa0809493.

15. Dell RM, Adams AL, Greene DF, Funahashi TT, Silverman SL, Eisemon EO, et al. Incidence of atypical nontraumatic diaphyseal fractures of the femur. Journal of Bone and Mineral Research. 2012;27(12):2544-50. https://doi.org/10.1002/jbmr.1719.

16. Du B, Daniels VR, Vaksman Z, Boyd JL, Crady C, Putcha L. Evaluation of physical and chemical changes in pharmaceuticals flown on space missions. The AAPS journal. 2011;13(2):299-308. https://doi.org/10.1208/s12248-011-9270-0.

17. Eastell R, Rosen CJ, Black DM, Cheung AM, Murad MH, Shoback D. Pharmacological Management of Osteoporosis in Postmenopausal Women: An Endocrine Society Clinical Practice Guideline. The Journal of Clinical Endocrinology & Metabolism. 2019;104(5):1595-622. https://doi.org/10.1210/jc.2019-00221.

18. Eyal S. How do the pharmacokinetics of drugs change in astronauts in space? Expert Opinion on Drug Metabolism & Toxicology. 2020;16(5):353-6. https://doi.org/10.1080/17425255.2020.1746763.

19. Friedberg W CK. Ionizing Radiation in Earth's Atmosphere and in Space Near Earth. Washington DC: Office of Aerospace Medicine, Federal Aviation Administration; 2011. Available from: http://www.tc.faa.gov/its/worldpac/techrpt/am11-9.pdf.

20. Geller M, Wagman R, Ho P, Siddhanti S, Stehman-Breen C, Watts N, et al. SAT0479 Early Findings from Prolia® Post-Marketing Safety Surveillance for Atypical Femoral Fracture, Osteonecrosis of the Jaw, Severe Symptomatic Hypocalcemia, and Anaphylaxis. Annals of the Rheumatic Diseases. 2014;73(Suppl 2):766-7. https://doi.org/10.1136/annrheumdis-2014-eular.1170.

21. Graebe A, Schuck EL, Lensing P, Putcha L, Derendorf H. Physiological, pharmacokinetic, and pharmacodynamic changes in space. Journal of clinical pharmacology. 2004;44(8):837-53. https://doi.org/10.1177/0091270004267193.

22. Harm DL, Sandoz GR, Stern RM. Changes in gastric myoelectric activity during space flight. Digestive Diseases and Sciences. 2002;47(8):1737-45. https://doi.org/10.1023/a:1016480109272.

23. Jones G, Nguyen T, Sambrook P, Kelly P, Eisman J. Progressive loss of bone in the femoral neck elderly people: longitudinal findings from the Dubbo osteoporosis epidemiology study. British Medical Journal. 1994;309:691-5. https://doi.org/10.1136/bmj.309.6956.691.

24. Karopoulou E, Augoulea A, Triantafyllopoulos I, Rizos D, Armeni E, Pliatsika P, et al. Comparative effects of denosumab or bisphosphonate treatment on bone mineral density and calcium metabolism in postmenopausal women. Journal of musculoskeletal & neuronal interactions. 2017;17(1):444. https://doi.org/10.1016/j.maturitas.2017.03.136.

25. LeBlanc A, Matsumoto T, Jones J, Shapiro J, Lang T, Shackelford L, et al. Bisphosphonates as a supplement to exercise to protect bone during long-duration spaceflight. Osteoporosis International. 2013;24(7):2105-14. https://doi.org/10.1007/s00198-012-2243-z.

26. LeBlanc AD, Driscol TB, Shackelford LC, Evans HJ, Rianon NJ, Smith SM, et al. Alendronate as an effective countermeasure to disuse induced bone loss. J Musculoskelet Neuronal Interact. 2002;2(4):335-43. http://www.ismni.org/jmni/pdf/8/LeBlanc.pdf.

27. Leder BZ, Wein MN, editors. Osteoporosis: Pathophysiology and Clinical Management. Switzerland: Springer Nature; 2020. Available from: https://link.springer.com/book/10.1007/978-3-319-69287-6.

28. Lewiecki EM, Blicharski T, Goemaere S, Lippuner K, Meisner PD, Miller PD, et al. A Phase III Randomized Placebo-Controlled Trial to Evaluate Efficacy and Safety of Romosozumab in Men With Osteoporosis. The Journal of Clinical Endocrinology & Metabolism. 2018;103(9):3183-93. https://doi.org/10.1210/jc.2017-02163.

29. Lipton A, Fizazi K, Stopeck AT, Henry DH, Brown JE, Yardley DA, et al. Superiority of denosumab to zoledronic acid for prevention of skeletal-related events: a combined analysis of 3 pivotal, randomised, phase 3 trials. European Journal of Cancer. 2012;48(16):3082-92. https://doi.org/10.1016/j.ejca.2012.08.002.

30. Miller PD, Bolognese MA, Lewiecki EM, McClung MR, Ding B, Austin M, et al. Effect of denosumab on bone density and turnover in postmenopausal women with low bone mass after long-term continued, discontinued, and restarting of therapy: a randomized blinded phase 2 clinical trial. Bone. 2008;43(2):222-9. https://doi.org/10.1016/j.bone.2008.04.007.

31. Miller PD, Wagman RB, Peacock M, Lewiecki EM, Bolognese MA, Weinstein RL, et al. Effect of Denosumab on Bone Mineral Density and Biochemical Markers of Bone Turnover: Six-Year Results of a Phase 2 Clinical Trial. The Journal of Clinical Endocrinology & Metabolism. 2011;96(2):394-402. https://doi.org/10.1210/jc.2010-1805.

32. Narici M, Boer M. Disuse of the musculo-skeletal system in space and on earth. European Journal of Applied Physiology. 2011;111(3):403-20. https://doi.org/10.1007/s00421-010-1556-x.

33. National Aeronautics and Space Administration. Human Research Roadmap 2021 [2021-Oct-11]. Available from: https://humanresearchroadmap.nasa.gov/.

34. Novartis. Aclasta Product Information 2022. Available from: http://www.guildlink.com.au/gc/ws/nv/pi.cfm?product=nvpaclin11115.

35. Organon Global Inc. Fosamax Product Information 2020. Available from: http://www.merck.com/product/usa/pi_circulars/f/fosamax/fosamax_pi.pdf.

36. Rosella D, Papi P, Giardino R, Cicalini E, Piccoli L, Pompa G. Medication-related osteonecrosis of the jaw: Clinical and practical guidelines. J Int Soc Prev Community Dent. 2016;6(2):97-104. https://doi.org/10.4103/2231-0762.178742.

37. Saag KG, Petersen J, Brandi ML, Karaplis AC, Lorentzon M, Thomas T, et al. Romosozumab or Alendronate for Fracture Prevention in Women with Osteoporosis. New England Journal of Medicine. 2017;377(15):1417-27. https://doi.org/10.1056/NEJMoa1708322.

38. Sibonga JD, Evans HJ, Sung HG, Spector ER, Lang TF, Oganov VS, et al. Recovery of spaceflight-induced bone loss: Bone mineral density after long-duration missions as fitted with an exponential function. Bone. 2007;41(6):973-8. https://doi.org/10.1016/j.bone.2007.08.022.

39. Sibonga JD, Matsumoto T, Jones J, Shapiro J, Lang T, Shackelford L, et al. Resistive exercise in astronauts on prolonged spaceflights provides partial protection against spaceflight-induced bone loss. Bone. 2019;128:1120-37. https://doi.org/10.1016/j.bone.2019.07.013.

40. Smith SM, Heer MA, Shackelford LC, Sibonga JD, Ploutz-Snyder L, Zwart SR. Benefits for bone from resistance exercise and nutrition in long-duration spaceflight: Evidence from biochemistry and densitometry. Journal of Bone and Mineral Research. 2012;27(9):1896-906. https://doi.org/10.1002/jbmr.1647.

41. Smith SM, Heer MA, Shackelford LC, Sibonga JD, Spatz J, Pietrzyk RA, et al. Bone metabolism and renal stone risk during International Space Station missions. Bone. 2015;81:712. https://doi.org/10.1016/j.bone.2015.10.002.

42. Smith SM, McCoy T, Gazda D, Morgan JLL, Heer M, Zwart SR. Space Flight Calcium: Implications for Astronaut Health, Spacecraft Operations, and Earth. Nutrients. 2012;4:2047-68. https://doi.org/10.3390/nu4122047.

43. Smith SM, Zwart SR, Heer M, Hudson EK, Shackelford L, Morgan JL. Men and Women in Space: Bone Loss and Kidney Stone Risk After Long-Duration Spaceflight. Journal of Bone and Mineral Research. 2014;29(7):1639-45. https://doi.org/10.1002/jbmr.2185.

44. Sosa-Henríquez M, Torregrosa O, Déniz A, Saavedra P, Ortego N, Turrión A, et al. Multiple vertebral fractures after suspension of denosumab. A series of 56 cases. International journal of clinical practice. 2021;75(10):e14550. https://doi.org/10.1111/ijcp.14550.

45. Stavnichuk M, Mikolajewicz N, Corlett T, Morris M, Komarova SV. A systematic review and meta-analysis of bone loss in space travelers. npj Microgravity. 2020;6(1):13. https://doi.org/10.1038/s41526-020-0103-2.

46. The Royal Australian College of General Practitioners, Osteoporosis Australia. Osteoporosis prevention, diagnosis and management in postmenopausal women and men over 50 years of age. East Melbourne, VIC: RACGP; 2017. Available from: https://www.racgp.org.au/getattachment/2261965f-112a-47e3-b7f9-cecb9dc4fe9f/Osteoporosis-prevention-diagnosis-and-management-in-postmenopausal-women-and-men-over-50-years-of-age.aspx.

47. Vico L, Hargens A. Skeletal changes during and after spaceflight. Nature Reviews Rheumatology. 2018;14(4):229-45. https://doi.org/10.1038/nrrheum.2018.37.

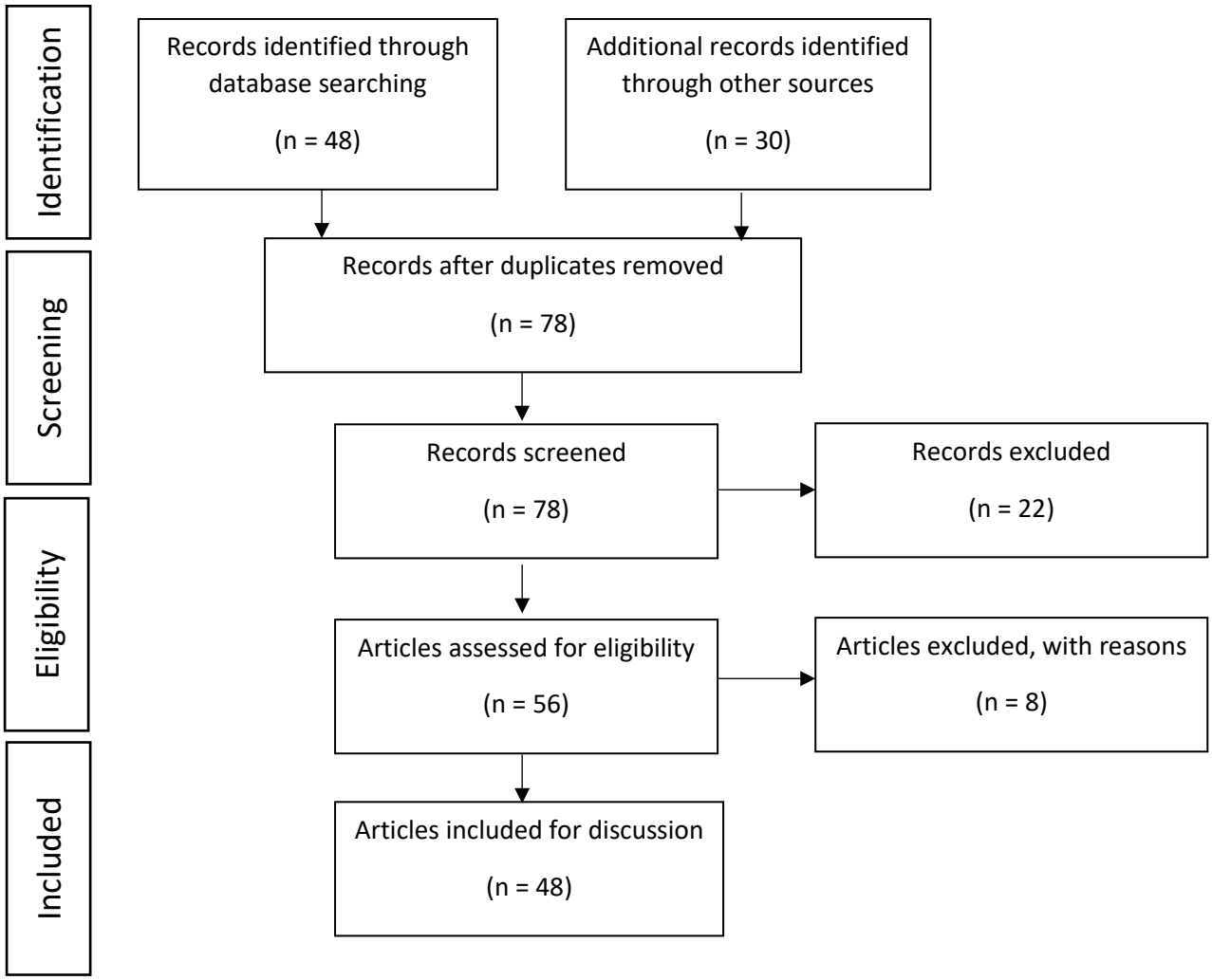
48. Zhang L, Pang Y, Shi Y, Xu M, Xu X, Zhang J, et al. Indirect comparison of teriparatide, denosumab, and oral bisphosphonates for the prevention of vertebral and

nonvertebral fractures in postmenopausal women with osteoporosis. Menopause. 2015;22(9):1021-5. https://doi.org/10.1097/gme.0000000000000466.

Table I – Comparison of alendronate and denosumab in spaceflight

	Alendronate	Denosumab
Advantages	• Multiple formulations (daily, weekly or monthly) • No special storage requirements • Cheap • Proven in spaceflight	• Six monthly dosing via subcutaneous injection • Superior relative fracture risk reduction and BMD maintenance • No GIT side effects • Lower risk of osteonecrosis and atypical femur fractures
Disadvantages	• GIT side effects common • Administered upright (not possible in microgravity) • Interactions with dietary calcium and magnesium	• Expensive • Requires refrigeration • No spaceflight data • Increased vertebral fracture risk on discontinuation (rare) • Risk of hypocalcaemia (rare)

Figure I – Article selection flowchart



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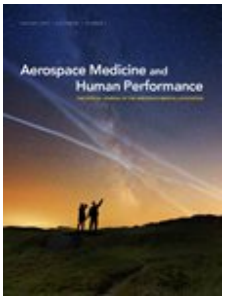

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