CLINICAL TRIAL PROTOCOL

Skeletal Outcomes Following Intensive Care

(SOFter)

Effect of denosumab on bone turnover markers in critically ill women - A safety and feasibility, randomised, placebo controlled trial

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1. GENERAL INFORMATION

Protocol Title: Effect of denosumab on bone turnover markers in critically ill women - A safety and feasibility, randomised, placebo, controlled trial

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2. SYNOPSIS

Background: Intensive care patients face health issues that extend beyond their critical illness. The current evidence indicates an association between critical illness and skeletal morbidity. This includes increased loss of bone mineral density (BMD), increased bone turnover markers (BTMs), increased fracture risk, and an increased rate of fragility fracture compared to matched community controls. This is most pronounced in older female survivors of critical illness. Bone antiresorptive therapies are effective at reducing bone loss, decreasing fracture risk, and may reduce mortality in patients with osteoporosis. A recent retrospective cohort study described an association between concurrent antiresorptive therapy and reduced mortality in critical illness[1]. Denosumab is a human monoclonal antibody directed against RANKL, a central stimulator of osteoclast activity, and is effective for prevention of fractures and bone loss in osteoporosis, and malignancy, with evidence of superiority compared to bisphosphonates. It is metabolised by intracellular mechanisms, with no adjustment necessary in renal dysfunction. No prospective randomised controlled studies have described the effect of antiresorptive therapies on long-term bone or mortality outcomes in critically ill patients.

Hypotheses: The administration of denosumab to critically ill postmenopausal women will safely and effectively attenuate critical illness associated bone loss.

Objectives:

- **Primary Objective:** Assess the efficacy and safety of subcutaneous denosumab in postmenopausal intensive care patients requiring longer than 24 hours of mechanical ventilation
- **Secondary Objectives:** Obtain early feasibility and biochemical efficacy data for a subsequent phase IIb study

Methods: A prospective, randomised, controlled, trial of denosumab (60mg sc 6-monthly) compared to placebo, in post-menopausal female intensive care patients requiring longer than 24 hours of mechanical ventilation. A sample size of 18 participants has been chosen to determine a clinically significant effect on bone turnover markers.

Significance: The role of antiresorptive therapies, including denosumab, in survivors of critical illness, to prevent bone loss, fracture, or death, requires an initial program of testing for safety and efficacy. The evidence from this trial will be used to inform progress to larger trials with bone mineral density, fracture, and mortality as the primary outcome.

3. BACKGROUND AND RATIONALE

3.1 Introduction

Intensive care patients face health issues that extend beyond their critical illness. Compared to their pre-illness status and general population controls, survivors of critical illness face increased mortality[2-5], physical[2,6-8] and cognitive impairment[9-11], and psychological distress[12-14]. A specific area where critical illness may adversely affect the well-being of survivors relates to an increased risk of fragility fracture due to accelerated bone loss[15-18]. Osteoporosis is a chronic progressive disease and major public health issue[19], characterized by low bone mass, micro-architectural bone disruption, and skeletal fragility leading to fracture[20]. The lifetime risk of osteoporotic spine, hip, or wrist fracture is 30-40% in developed countries, and the lifetime risk of hip fracture is one in six in white females[21], with significant associated health burden of mortality, morbidity, and cost[22,23]. However, as few as 13-27% of patients with osteoporosis are treated following a fragility fracture, suggesting osteoporosis remains an under diagnosed disease[24,25].

3.2 Pathophysiology of osteoporosis

Normal bone turnover requires osteoclast and osteoblast activity to be tightly coupled, with regulation by mechanical, nutritional, immune, paracrine, autocrine and endocrine factors [9,7,8]. This modeling and remodelling results in changes to the size and contours of bone internally and externally, a normal process that establishes bones peak strength during growth, and works to maintain it during aging. Remodelling, resorption, then replacement, occurs asynchronously through the skeleton, and involves 5-10% of the skeleton per year[21]. The replication, differentiation, activity, and lifespan of osteoclast and osteoblast progenitors are determined by growth factors from matrix, cytokines, circulating hormones, soluble and membrane-bound products of osteoclasts and their precursors, signals from osteocytes, and immune cells from osteoblast lineage. Osteoclasts are derived from haemopoietic precursors from the capillary blood supply and marrow, and are closely related to macrophages. Differentiation from osteoclast precursor to mature osteoclast requires signals from macrophage-colony-stimulating factor (M-CSF), receptor activator of nuclear factor-κB ligand (RANKL), and vascular endothelial growth factor (VEGF). RANKL is abundantly expressed by osteoblasts, bone marrow stromal cells, and T and B-lymphocytes, and binds to RANK receptor on osteoclasts, stimulating activity. Osteoblasts also release osteoprogeretin, a RANKL decoy/ antagonist. Osteoblasts are stimulated by vitamin D, parathyroid hormone, and the development of mature osteoblasts is promoted by growth factors released from bone matrix during resorption, and produced by osteoblasts themselves. Many of these local factors also contribute to osteoblast and osteoclast apoptosis. Uncoupling of bone resorption and formation occurs in numerous conditions, including menopause, myeloma, rheumatoid arthritis, bone metastases, suppression of sex hormones (androgen suppression therapy for prostate cancer in men, aromatase inhibitor therapy for breast cancer in women), and in the presence of pro-inflammatory cytokines (IL-1, TNF)[26]. Oestrogen deficiency increases the rate of remodelling and the volume of bone resorption by prolonging the life span of osteoclasts, and decreasing the life span of

osteoblasts. This leads to trabecular thinning, loss of connectivity between trabeculae, cortical thinning, and increased cortical porosity. As a result bone fragility is more common in women than men, partly because the production of sex hormones does not decrease rapidly in men, with no subsequent increase in remodelling rate. The bone fragility and fractures observed in osteoporosis vary in pathogenesis, with some related to reduced bone mineral density, others a reduced density of osteocytes, and high, normal, or low rates of remodelling.

3.3 Assessment of Bone

Bone Mineral Density

The measurement of BMD by dual energy x-ray absorptiometry (DXA) at the proximal femur and lumbar spine forms the basis of assessment and treatment of osteoporosis, with change in BMD estimated to account for 60-80% of variance in bone strength[19], and is the central component of internationally agreed definitions of osteoporosis [27]. BMD values in individuals are expressed as an absolute value (g/cm^2), and in relation to a reference young adult population in standard deviation (SD) units, the T-score. The T-score is the number of standard deviations above or below the young adult mean, with cut-off values calculated from the Australian reference ranges[28,29]. The WHO operational definition[30] of osteoporosis includes normal (T-score > -1.0), osteopaenic (T-score -2.5 to -1.0), or osteoporotic (T-score <-2.5). Established osteoporosis is defined as a T-score below -2.5 in the presence of one or more fragility fractures [20]. BMD measurement is also used to estimate fracture risk, providing a continuous relationship with no absolute cut-off threshold that discriminates who will and will not fracture. Individuals with a 1SD decrease in BMD compared to their age-matched peers will have an approximate 2-fold increase risk of fractures in their remaining lifetime. This increases to 4-fold increase in fracture risk for a T-score of -2.5 [18]. In addition to categorisation of osteoporosis, BMD is used to assess response to treatment, and as a surrogate outcome in trials of antiresorptive agents. Change in BMD over one year is the standard for interventional research studies[31-35], as BMD undergoes relatively small changes over time, of a magnitude similar to measurement error (short-term precision in vivo for Lunar DXA (GE Healthcare, Madison, USA) is 1.6% for the femoral neck and 0.6% for the lumbar spine[1]).

Bone Turnover Markers

Biochemical markers of bone turnover also have a role in the assessment of bone loss. Although the diagnosis of osteoporosis is not based on evaluation of biochemical markers, they are used in predicting the rate of bone loss and subsequent fracture risk[36,37]. Overall BTMs are separated into markers of bone resorption and bone formation [38]. The bone resorption markers include urinary collagen type 1 cross-linked N-telopeptide (NTX), pyridinoline (Pyd) or deoxypyridinoline (Dpd), carboxy-terminal cross-linked telopeptide of type 1 collagen (ICTP/CTX). Bone formation markers include skeletal alkaline phosphatase (SALP), osteocalcin (OC), procollagen type 1 C peptide (P1CP) and procollagen type 1 N peptide (P1NP). The cytokine receptor osteoprotegerin (OPG), a member of the TNF receptor superfamily, acts as a decoy receptor for receptor activator of nuclear factor kappa B ligand (RANKL), and prevents RANK mediated regulation of inflammation, innate immunity, apoptosis, and blocking maturation and activity of osteoclast

precursors. Although divided into formation and resorption markers, BTM levels are affected by a number of factors, requiring more complex interpretation. The bone formation markers P1NP and P1CP are both procollagen terminal extension peptides, but P1NP is more specific for bone formation. Also a number of BTMs are affected by biological factors including age, gender, co-existing disease, and medications. Examples include decreased excretion of CTX in renal failure and sensitivity of OC to glucocorticoid exposure [38]. Markers for bone turnover are generally higher in those with osteoporosis compared to healthy controls, although there is considerable overlap. The combined use of BMD measurement and biochemical markers may be helpful in risk assessment, especially in those women who are not identified as at risk by BMD measurement alone [23]. Levels of bone markers decrease rapidly with antiresorptive therapies, with 30-60% decreases after 3-6 months. The short-term decrease in bone markers predicts the effects of antiresorptive agents on bone mass and fracture risk over the subsequent 2-year, thus providing a useful measure of treatment efficacy [24].

3.4 Consequences of osteoporosis

The consequences of fragility fractures are devastating in terms of mortality, morbidity, and cost[22,23]. Three-quarters of women with hip, pelvis, or lower limb fractures are confined to the home, or could walk only short distances for several weeks. After a year, nearly one-half have not regained pre-fracture mobility. One-seventh of women with upper-limb fractures did not venture outside the home for at least 6 weeks. After 6 months, 3.4% of all patients, 19.6% of hip, 12.8% of humeral, and 4.7% of spine fracture patients required assistance with bathing and showering. After a year, more than half of the hip fracture cases remained restricted regarding housework, gardening, and transport. In summary, a fracture, regardless of site, has a major impact on a woman's lifestyle and well-being for at least a year [22]. Despite the known consequences, as few as 13-27% of patients with osteoporosis are treated following a fragility fracture, suggesting osteoporosis remains an under diagnosed disease[24,25].

The consequences of osteoporosis extend to mortality. Between 10 to 20% of people who sustain a hip fracture die within one year[21], the risk highest in the first six-months and decreases over time. However, the relative contribution of fracture, comorbidity, or other mechanisms to subsequent mortality is disputed [21]. In addition, this association is strengthened by the relationship between osteoporosis treatments and reduced mortality. A meta-analysis of RCTs of studies investigating approved doses of medication with proven efficacy in preventing vertebral and non-vertebral fractures, with a duration of at least 12 months and reporting mortality, identified eight studies of four agents (risedronate, strontium ranelate, zoledronic acid, and denosumab), providing data of over 1400 deaths in approximately 40,000 subjects. Overall osteoporosis treatment was associated with an 11% reduction in mortality (RR 0.89, 95%CI 0.80-0.99, p=0.036)[39]. Meta-regression analyses revealed mortality reduction was not related to mean age, incidence of hip or non-vertebral fracture in the placebo group, or non-vertebral fracture risk reduction, but was associated with the baseline mortality rate of the placebo group (P=0.03). In the four studies where the placebo mortality rate was greater than 10 per 1000 patient years (range 13.9-70.2 deaths per 1000 patient-years), there was a significant reduction in mortality (RR 0.83; 95% CI 0.72-0.94, p=0.0052), compared to no reduction in

mortality in studies where placebo mortality rate was less than 10 per 1000 years (RR 1.01, 95% CI 0.87-1.19, p=0.86) [39]. The mortality effect appeared to be similar across the different classes of agents in the study.

3.5 Bone loss following critical illness

The current evidence of association between critical illness and accelerated bone loss includes changes in bone mineral density (BMD), bone turnover markers (BTMs), fracture risk, and fragility fracture rate.

Bone turnover markers and critical illness

A number of studies have identified a relationship between critical illness requiring mechanical ventilatory support and increased bone turnover, summarised in a recent systematic review[16] . Increased osteoclastic bone resorption (increased urinary DpD and PyD, serum CTX/ICTP), an increase in immature osteoblast number and activity (serum P1CP and P1NP), and reduced activity of mature osteoblasts (serum OC and ALP), of the magnitude described in postmenopausal females, or metabolic bone disease have been described[17,37,40,41]. Higher levels of bone resorption markers were observed in ICU patients with a length of stay of greater than 5-days, and a positive relationship between inflammation and increased bone turnover was present in a number of studies and was unrelated to severity of illness, type of illness, age or outcome.

There is limited evidence describing the effect of known osteoporosis risk factors and critical illness related factors on BTMs in critical illness, with the exception of age and gender. Higher levels of bone resorption markers were observed in ICU patients with a length of stay of greater than 5-days [42], although the lack of adjustment for confounders, including co-morbid illness such as renal failure, prevents the nature of this relationship being established. A positive relationship between inflammation and increased bone turnover was present in a number of studies [40,43-45], and was unrelated to severity of illness, type of illness, age or outcome. Systemic inflammation has been identified as a marker for increased fracture risk in non-critically ill patients [46], however ongoing bone resorption did not correlate with inflammatory markers, which may reflect the influence of other mechanisms, a prolonged effect of cytokines through osteoclast activation factors that increase maturation and lifespan of osteoclasts, or a direct effect of cytokines on osteoclast precursors. In one of the studies, concomitant treatment with glucocorticoids, thyroid hormones, or any other ICU medication did not significantly affect markers of bone turnover at any of the studied time points [43-45]. A series of studies by Van den Berghe et al [43,44] described changes to the somatotrophic, thyrotrophic, and gonadotrophic axes in prolonged critical illness, and included bone markers as a part of measures of target tissue effects. The studies describe a positive correlation between inflammatory cytokines and osteoclastic and osteoblastic activity, with variable effects of restoration of somatotrophic, thyrotrophic, and gonadotrophic axes on BTMs [47]. In-vitro experiments have shown that compared to healthy controls, critically ill patients peripheral blood mononuclear cells (PBMCs) responded to the presence of osteoclastic activation factors with an increased number and activity of mature osteoclasts [18]. In addition, exposure of PBMCs to critically ill patient sera resulted in an increased formation of mature osteoclasts, whereas a model of bone formation showed a reduction in angiogenesis factor expression, and reduced vascularity and maturity of

bone formation.

Bone mineral density assessment and critical illness

To date there are two prospective observational studies describing longitudinal changes in BMD in survivors of critical illness. The first described changes in calcaneal BMD over 10-days in 46 adult patients expected to be ventilated for over 48 hours and remain in ICU for over 7-days. They reported a decrease in BMD ARDS patients compared to ventilated non-ARDS patients (-2.81% vs +2.40%, p=0.03)[18], and an increase in fracture risk of 19.4% in ARDS compared to 9.35% in non-ARDS patients (p=0.012). The use of calcaneal BMD limited by precision issues, the short measurement period, and small numbers are major limitations to this study.

The second study describes the change in BMD in the year after critical illness in 66 adult patients ventilated for greater than 24 hours who survived to ICU discharge[17]. The annual decrease in BMD in critical illness was significantly greater than age and gender matched population controls[48] (Table 2). When analysed by gender, the difference was significantly greater in females at both AP spine and femoral neck, while in males it was significantly greater at femoral neck only. This study also reported the percentage of patients with an osteoporotic or osteopaenic T-score and fracture risk. The proportion of patients with abnormal T-score at 1-year post ICU (females 66.7%, males 44.1%) were higher than local population levels, with the Geelong Osteoporosis Study (GOS) reporting one-fifth of females greater than fifty years of age have BMD in the osteopaenic range, and 1 in 6 with osteoporosis[49].

Table 2: Table 1: Annualised change in bone mineral density in women after critical illness compared to matched Geelong Osteoporosis Study controls (Data are shown as mean (±standard deviation))

Variable	ICU (n=31)	GOS (n=120)	Difference (95% CI)	P-value
Total change AP spine	-0.035 (0.050)	-0.002 (0.012)	-0.033 (-0.042, -0.023)	< 0.001
Percent change AP spine	-2.85 (4.05)	-0.18 (1.08)	-2.67 (-3.49, -1.86)	< 0.001
Total change Femur	-0.018 (0.037)	-0.006 (0.008)	-0.013 (-0.020, -0.005)	0.001
Percent change Femur	-1.96 (4.03)	-0.65 (0.98)	-1.31 (-2.10, -0.51)	0.001

This study also calculated fracture risk using the Australian version of the FRAX® fracture risk assessment tool, an algorithm developed by the World Health Organization (WHO)[50]. The estimated 10-year fracture risk for both all major fractures (4.85±5.25 vs 5.50±5.52, p<0.001) and hip fractures specifically (1.57±2.40 vs 1.79±2.69, p=0.001) significantly increased, and was highest in females.

Fragility fractures in survivors of critical illness

The major sequelae of increased bone turnover, and accelerated bone loss, is an increased risk of fragility fracture. The fragility fracture rate following critical illness, and comparison to age and gender matched population controls, has been described in one retrospective observational case-cohort study [15]. The radiological databases of 739 adult patients that were ventilated for greater than 24 hours and survived to ICU discharge, were assessed for evidence of fragility fracture using the same ascertainment period as the

control population, the GOS [48]. In the ICU survivor cohort followed for a median of 3.7 years, thirty-six women (14.2%) and 48 men (10.0%) sustained a fracture during the post-ICU time period, and incident fracture rate of 3.84 and 2.41 per 100 patient-years respectively. The over 60-year female ICU survivor cohort were compared to the GOS gender and age matched controls, with a significant increase in fracture, and shorter time to fracture observed in in the ICU group (HR 1.65 95%CI 1.08-2.52) (p = 0.02).

Figure 2: Unadjusted and adjusted fracture rates and hazard ratios for females (20-94 yrs of age) post-ICU compared with population-based females (GOS)

Variable	Post-ICU Fracture Rate (95% CI)	GOS Fracture Rate (95% CI)	Unadjusted HR (95% CI)	Adjusted HR (95% CI, *p*)
All ages, all fracture	3.84 (2.58–5.09)	2.01 (1.76–2.25)	1.63 (1.14–2.32)	1.20 (0.84–1.71, $p = .31$)
>60 yrs of age, osteoporotic fracture	4.33 (2.72–5.93)	2.81 (2.33–3.28)	1.48 (0.98–2.25)	1.65 (1.08–2.52, $p = .02$)

ICU, intensive care unit; GOS, Geelong Osteoporosis Study; CI, confidence interval; HR, hazards ratio.

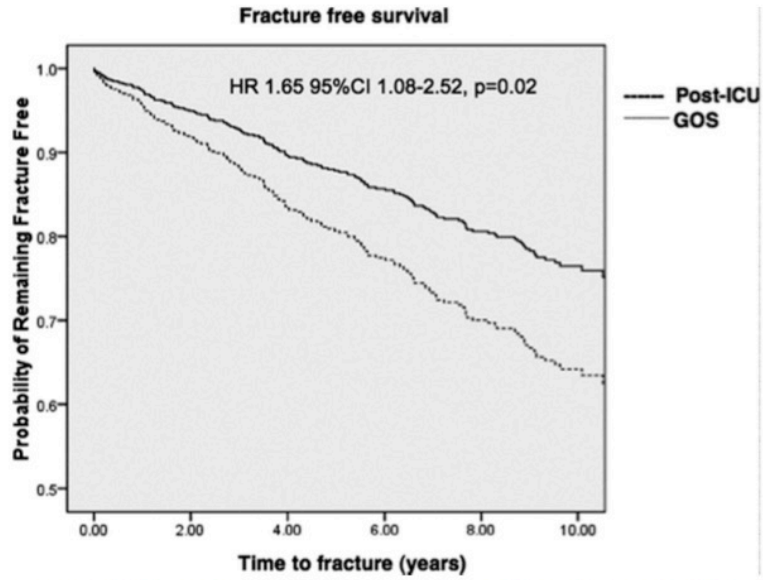


Figure 1. Time to fracture of the wrist, hip, humerus, or vertebral fracture after intensive care unit (*ICU*) compared with the random population-based sample in older age group (≥ 60 yrs) females. *HR*, hazard ratio; *CI*, confidence interval; *GOS*, Geelong Osteoporosis Study.

3.6 Prevention of critical illness related bone loss

The evidence to date supports the hypothesis that bone loss is increased during critical illness, resulting in an increased risk of fracture in survivors. This would contribute significantly to their health burden; with the average cost of hip fracture in Australia is estimated at $16,000, with an average length of hospital stay of thirteen days [10]. Furthermore, fragility fractures are associated with excess mortality, pain, immobility, and reduced functional capacity resulting in significant quality of life issues [12 16 17 11]. To date there is no evidence

of an association between accelerated bone turnover and increased mortality after critical illness. The availability of target interventions to prevent or attenuate acute bone loss following critical illness provides the incentive to further explore this area of clinical research. The management of osteoporosis can be classified into non-pharmacological options, with pharmacological treatments classified as ant-resorptive and anabolic.

Non-pharmacologic options – Physical Activity and Modifiable Risk Factors

Physical activity, including resistance and weight-bearing exercise, can increase muscle mass and transiently improve BMD [51], and regular physical activity may result in beneficial effects on skeletal microarchitecture [52]. The relationship between falls and fractures is well described, with falls, and fractures from falls, increasing with age. Exercise and balance programs that result in reduced falls may be of benefit. Other measures that may be of benefit are reductions in known risk factors for reduced BMD, ie alcohol, smoking.

Calcium and Vitamin D

The efficacy of calcium and vitamin D treatment for the prevention of osteoporotic fractures in controversial, with conflicting results from large trials, subgroup analyses, and meta-analyses. Standard recommendations for most postmenopausal women with osteoporosis suggest a total calcium intake of 1000-1500mg per day, and a total vitamin D intake of 600-800 IU per day [53].

The association between serum vitamin D levels and outcomes in critically ill patients has received attention since the publication in 2009 of a case series describing a high prevalence of hypovitaminosis D in 42 critically ill patients referred to an endocrinology service[54]. With an association between vitamin D deficiency and increased mortality present in the general community and specific disease cohorts[55,56], and a plausible mechanism for vitamin D to influence outcomes through its non-bone related activity in endothelial, immune, and cellular function [57-60], the links between vitamin D as both a prognostic marker and intervention in the critically ill population has been of increasing interest. Although there is debate regarding the threshold levels used to define insufficiency and deficiency, the proportion of critically ill patients with decreased vitamin D levels ranges from 42-97%[61-70 71]. A positive association between vitamin D deficiency during critical illness and increased mortality has been described in observational studies where cohorts of patients with vitamin D levels measured before or during critical illness were examined [62,66,69,72,73]. These studies consistently describe increased mortality rates in vitamin D deficient patients, but are limited by the selection bias created by enrolling patients in whom vitamin D levels were already ordered. In comparison, six prospective observational cohort studies enrolling patients with predicted or actual ICU length of stay of greater than 1 to 2 days have reported conflicting results. A positive association between vitamin D deficiency and increased 90-day mortality has been reported in two studies [61,74], while no association was found in four studies reporting ICU, hospital, or 28-day mortality [70,71,75,76]. These results, in combination with evidence that vitamin D deficiency during critical illness is associated with increasing age, seasonal variation, severity of illness, bacteraemia, sepsis, multi-organ failure, type of ICU and length of stay [61,63,66,69,74-7619], suggest the association between critical illness, vitamin D deficiency, outcomes, and the effect of other factors, is not

clear.

In terms of bone turnover, two studies report the effects on bone turnover of treating vitamin D deficiency in critically ill patients. One study described the effect of parenteral vitamin D 200 IU or 500 IU daily in long-term surgical ICU patients receiving parenteral nutrition, with higher dose vitamin D associated with a relatively small increase in serum OC, a decrease in serum B-CTX, but did not affect other BTMs. In addition the decrease in inflammatory markers interleukin-6 and C-reactive protein over time was more pronounced with the higher dose vitamin D[40]. However treating vitamin D deficiency with calcitriol did not lead to a reduction in bone resorption markers, suggesting that vitamin D deficiency alone was not the mechanism for accelerated bone turnover[77].

Antiresorptive agents – Bisphosphonates

Bisphosphonates inhibit bone resorption in a dose dependent manner, and result in an increase in bone mass. Large prospective trials of osteoporotic women demonstrated increases in lumbar spine and femoral BMD over 2-3 years, and reduced vertebral, wrist and hip fracture risk. Multiple agents are available including etidronate, alendronate, clodronate, pamidronate, and zoledronic acid. They have poor oral bioavailability, with 1-5% of the oral dose absorbed. PBS indications for bisphosphonates include treatment for osteoporosis in a patient aged 70 years of age or older with a T-score of -3.0 or less, and treatment for established osteoporosis in patients with fracture due to minimal trauma.

Common side effects of oral bisphosphonates include fatigue, anaemia, muscle aches, fever, swelling feet or legs, and oesophageal and upper gastrointestinal irritation. Flu-like symptoms are common after intravenous infusions in treatment naïve individuals and are thought to occur because of their potential to activate human gamma delta T cells. The association between bisphosphonates and renal dysfunction is well established. Acute tubular necrosis and collapsing focal segmental glomerulosclerosis have been implicated in the mechanism of renal toxicity, however the pathogenesis is poorly understood. A review of the FDA Adverse Event Reporting System identified 72 cases of renal failure associated with zoledronic acid. Indications for use were multiple myeloma (42), solid tumours (22), benign conditions (2), and unknown condition (6). Renal failure developed after an average of 56 days of use, in 25% of patients only one dose was received. The onset of renal failure and recovery of serum creatinine after drug discontinuation suggested a temporal relation to the use of zoledronic acid. The authors recommended renal function monitoring, adequate hydration, and discontinuation if renal function deteriorates.[27] A rare complication is osteonecrosis of the jaw, with an estimated incidence of <1:10,000 bisphosphonate users[53], and mainly observed in multiple myeloma patients with zoledronate who have had dental extractions where the rate may be as high as 1 in 10 [28].

There is limited experience with bisphosphonates in critical illness. Case reports and small studies [8] have reported the use of intravenous bisphosphonates to treat critically ill patients with biochemical evidence of bone resorption. A single randomised controlled trial reported a transient decrease in serum CTX in chronic critically ill patients receiving a single intravenous dose of ibandronate compared to placebo [78]. A single randomised controlled trial has reported the effect of a single intravenous dose of ibandronate compared to

placebo, on serum CTX and OC over 14-days, in 20 postmenopausal chronic critically ill women[78]. Although ibandronate was associated with a significant decrease in CTX from baseline at day-6 compared to placebo (-34% vs +13%, p=0.03), this effect had disappeared by day-11. In comparison there were no differences in OC levels between the groups. This suggests ibandronate had a significant but short-lived effect on osteoclast activation and bone resorption, but was ineffective at suppressing osteoblast activation and bone formation. This is different to the effect observed in post-menopausal women, where reduction of CTX and OC or P1NP is attributed to treatment resulting in coupling of resorption and formation [79].

A retrospective analysis compared 245 patients with an ICU length of stay of at least 24 hours receiving bisphosphonates within 5-years prior to admission, to propensity matched ICU controls, for the association between prior bisphosphonate use, mortality, and change in vertebral BMD assessed by serial CT scans. They reported recent bisphosphonate use in 3.1% of eligible patients, with a significantly reduced morality in this group compared to matched controls (mortality RR 0.41, 95% CI 0.24-0.71, p<0.01). This relationship persisted after adjustment for known confounders of sex, age, premorbid disease burden, bisphosphonate route and time between ICU admission and bisphosphonate prescription. The only group in whom benefit disappeared were patients free of any comorbid disease. Serial CT assessment of vertebral BMD revealed lower baseline bone density in bisphosphonate users, with an attenuated decrease in BMD in users vs non-users (-3 $\pm$ 13% vs -15 $\pm$ 14% per week, p<0.01), over a short time period (11 $\pm$ 10 days).

Antiresorptive agents – Denosumab

Denosumab is a fully human monoclonal antibody directed against RANKL, a central stimulator of osteoclast activity. It is administered as a subcutaneous injection and is metabolised by intracellular mechanisms, with no adjustment necessary in renal dysfunction. Denosumab has been extensively trialed and shown to be effective at reducing loss of BMD and fracture prevention. It currently has indications for the prevention of skeletal-related events in bone metastases from solid tumors, treatment of androgen deprivation induced bone loss in men with prostate cancer, and treatment of aromatase inhibitor induced bone lose in women with breast cancer [80 81 82 80 83]. Although head-to-head trials of antiresorptive agents are lacking, denosumab appears to be at least as efficacious as other agents, and has the added advantage that is administered as a subcutaneous injection 6-monthly. This may improve compliance with antiresorptive therapy, a major issue for bisphosphonate therapy [84].

In clinical studies, treatment with 60 mg of denosumab resulted in reduction in the bone resorption marker CTX by 86% at 1-month post intervention compared to placebo. At 6-months, prior to the next scheduled dose, CTX reductions were partially attenuated with a mean reduction of 72% compared to placebo, reflecting the reversibility of the effects of denosumab on bone remodelling. These effects were sustained with continued treatment to 36-months [80]. In the same study P1NP was reduced 18% compared to placebo at 1-month, and 50% compared to placebo at 6-months, consistent with the physiological coupling of bone formation and resorption in skeletal remodelling.

Figure 3: Percent changes in BMD and Bone Turnover Markers for denosumab and placebo in post-menopausal women.

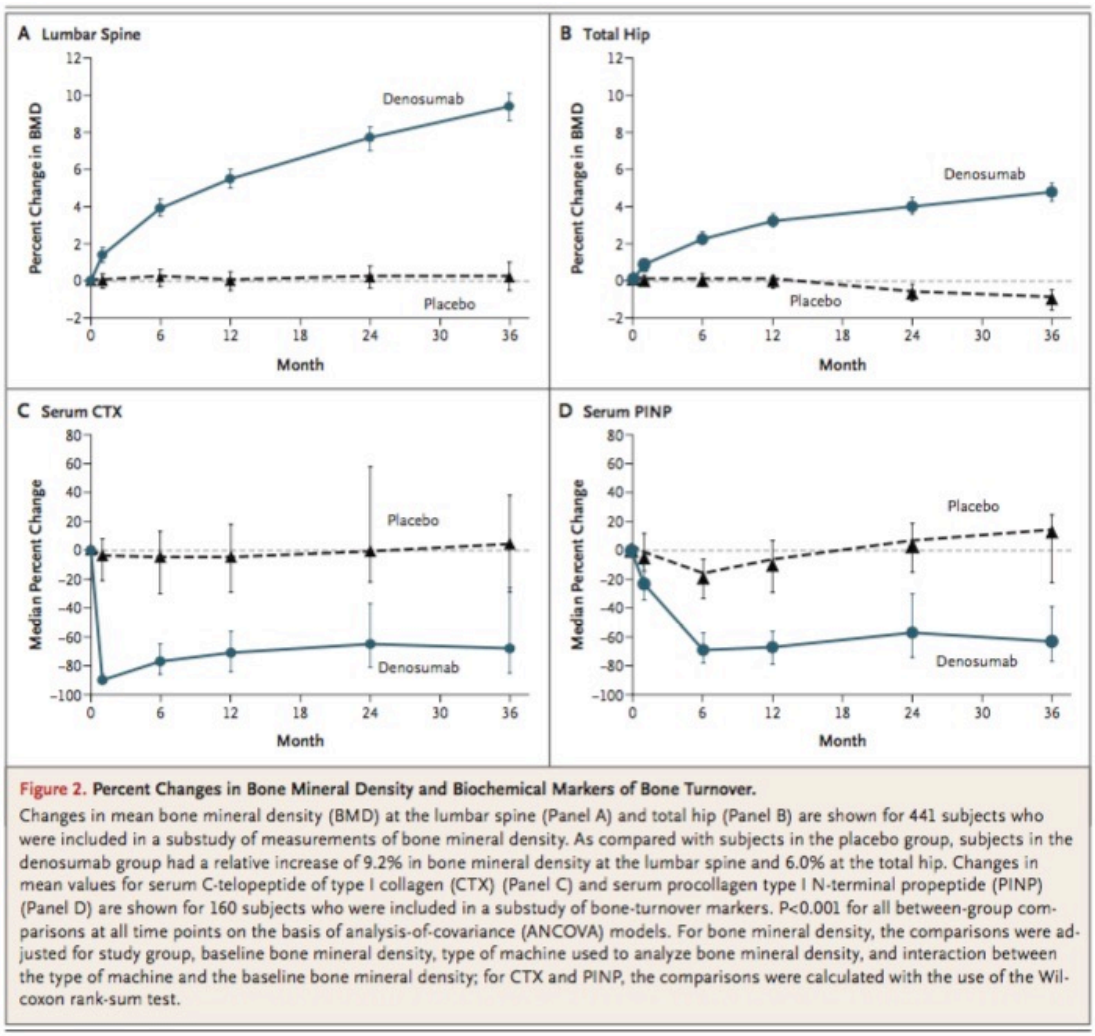


Figure 2. Percent Changes in Bone Mineral Density and Biochemical Markers of Bone Turnover.
Changes in mean bone mineral density (BMD) at the lumbar spine (Panel A) and total hip (Panel B) are shown for 441 subjects who were included in a substudy of measurements of bone mineral density. As compared with subjects in the placebo group, subjects in the denosumab group had a relative increase of 9.2% in bone mineral density at the lumbar spine and 6.0% at the total hip. Changes in mean values for serum C-telopeptide of type I collagen (CTX) (Panel C) and serum procollagen type I N-terminal propeptide (PINP) (Panel D) are shown for 160 subjects who were included in a substudy of bone-turnover markers. P<0.001 for all between-group comparisons at all time points on the basis of analysis-of-covariance (ANCOVA) models. For bone mineral density, the comparisons were adjusted for study group, baseline bone mineral density, type of machine used to analyze bone mineral density, and interaction between the type of machine and the baseline bone mineral density; for CTX and PINP, the comparisons were calculated with the use of the Wilcoxon rank-sum test.

(Cumming et al, NEJM, 2009;361(8);756-765)

Similar to all antiresorptive agents, adverse effects of denosumab include fatigue, headache, rash, musculoskeletal pain, hypocalcaemia, hypophosphatemia, and atypical fractures of the femoral shaft with long-term use. Hypocalcemia must be corrected prior to initiating therapy, and in patients predisposed to hypocalcemia and disturbances of mineral metabolism (e.g. history of hypoparathyroidism, thyroid surgery, parathyroid surgery, malabsorption syndromes, excision of small intestine, severe renal impairment [creatinine clearance < 30 mL/min] or receiving dialysis), clinical monitoring of calcium and mineral levels (phosphorus and magnesium) is highly recommended within 14 days of injection. Osteonecrosis of the jaw has been reported, but is rare, with no cases in 3420 cancer patients enrolled in a RCT [83]. Osteonecrosis of the jaw (ONJ), which can occur spontaneously, is generally associated with tooth extraction and/or local infection with delayed healing. A routine oral exam should be performed prior to initiation.

Perhaps the major concern about long-term use of denosumab relates to its possible effects on the immune system, since RANKL is expressed not just on bone cells but also on immune cells. In a clinical trial of over 7800 women with postmenopausal osteoporosis, the incidence of infections resulting in death was 0.2% in both treatment groups, and the incidence of nonfatal serious infections was 3.3% in the placebo and 4.0% in the denosumab groups. Hospitalizations due to serious infections in the abdomen (0.7% placebo vs. 0.9% denosumab), urinary tract (0.5% placebo vs. 0.7% denosumab), and ear (0.0% placebo vs. 0.1% denosumab) were reported. Endocarditis was reported in no placebo patients and 3 patients receiving denosumab. Skin infections, including erysipelas and cellulitis, were reported more frequently in patients treated with denosumab (< 0.1% placebo vs. 0.3% denosumab, p=0.002) [80].

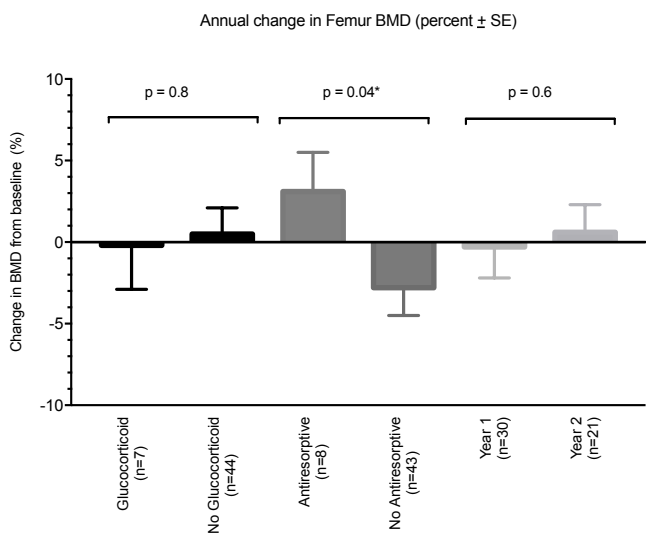
3.7 Denosumab as trial intervention in critical illness

The experience of antiresorptive medications in the critical care setting is limited to case reports and small cohort studies. We have recently reported on the association between antiresorptive agents (including alendronate, denosumab, strontium ranelate, and risedronate) on annual change in BMD in a cohort of men and women in the 2-years after critical illness. In women participants, a greater loss of spine BMD was observed in the first year after critical illness, with antiresorptive medication use associated with an increase in BMD compared to a decrease in BMD in those that did not receive such therapy. In men BMD loss increased in the second year after critical illness, and there was no association between use of antiresorptive medications or glucocorticoids and change in BMD, although only a small proportion of men received post-ICU bone-related medications. These findings suggest anti-resorptive therapy may be an effective intervention to prevent bone loss in women with critical illness, and prospective trials investigating this effect are warranted.[85] (Figure 2a and 2b – *Orford et al, Crit Care, under review)*.

Denosumab, with reduced renal effects, and efficacy, appears likely to be a more favourable target agent. Given the lack of experience in critical illness, the favourable characteristics of denosumab, and the existing evidence of accelerated bone loss in critical illness, this study proposes a safety and feasibility pilot, after which assessment of feasibility for a larger phase II trial could be considered.

This study proposes to enroll post-menopausal women requiring ventilatory support for greater than 24-hours, administer denosumab on day 3 in ICU, and again 6-months later. For the purpose of this safety and exploratory study the primary outcome will be change in the bone turnover markers CTX and P1NP to study day-28. Secondary outcomes include change in bone mineral density and bone turnover markers at 1-year post ICU, and safety outcomes.

Figure 2a: RMANOVA assessment of annual BMD change in women



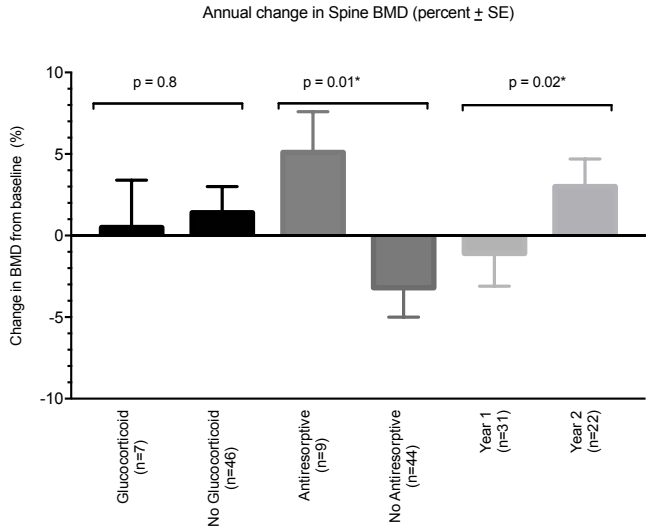
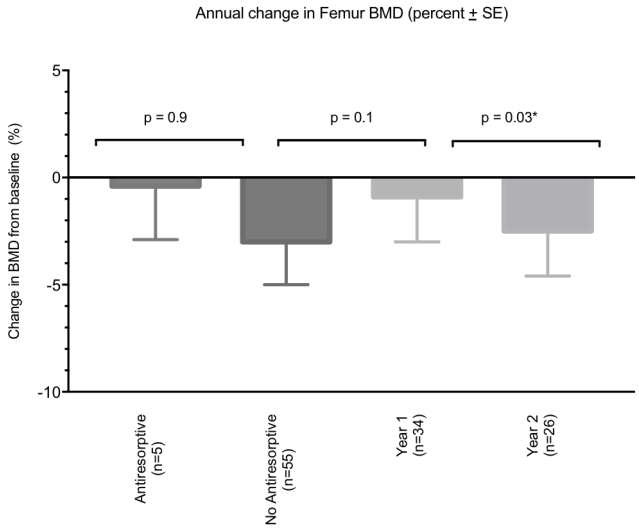
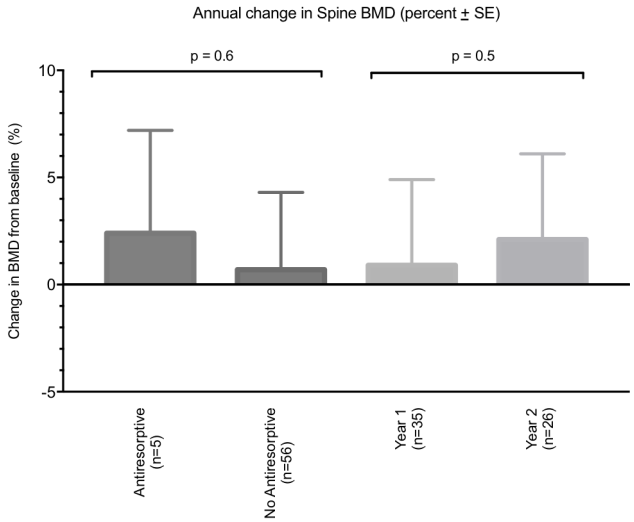


Figure 2b: RMANOVA assessment of annual BMD change in men





Administration of denosumab without prior BMD assessment

The indications for denosumab include postmenopausal women with osteoporosis at high risk of fracture, and treatment of bone loss in women receiving adjuvant aromatase inhibitor therapy for breast cancer. With regards to assessment of osteoporosis, DXA BMD testing cannot be performed in the ICU, because patients need sufficient mobility and cognitive function to transfer from a chair to a bed and lie still for the study. Our experience is this occurs one to four weeks after ICU discharge. Therefore, the intervention options are to administer denosumab in ICU without BMD testing, or to delay administration to the post-ICU period after BMD testing has been performed. The rationale for administering denosumab during ICU is three-fold;

1. The available evidence for accelerated bone turnover associated with critical illness indicates bone turnover markers increase within 48-hours of ICU admission, suggesting earlier intervention is more likely to be effective.

2. Our observational data revealed that 67% of female survivors of critical illness able to complete the 1-year follow-up had ostepaenia or osteoporosis. The cohort that withdrew or died before this had higher BTMs during ICU, suggesting we observed cohort that completed the study where healthier with lower risk of accelerated bone loss. Given this, it is estimate that less than 1/3 of women enrolled will have normal bone mass. General population data tells us that only a quarter of fragility fractures occur in women with osteoporosis, with ¾ occurring in women with osteopaenia and normal bone mass[17,49].

3. The administration of denosumab to postmenopausal women with a risk factor for accelerated bone loss irrespective of BMD has been performed in a 3500 patient randomised trial of women commencing an aromatase inhibitor for the management of breast cancer. In this study 55% of women enrolled had a BMD $\geq$ -1.0, and a significant reduction in fracture was observed with denosumab equally for women with normal and ostepaenic BMD. In addition, the change in BMD observed in the first year of the study was -1.81% (placebo) vs + 3.94% (denosumab) at lumbar spine, and -1.08% vs +2.29% at femur[83]. In comparison to the placebo group in this trial, female ICU survivors have a change in BMD of -2.85 $\pm$ 4.05% at lumbar spine and -1.96 $\pm$ 4.03% at femur.

Administration of denosumab and possible immune modulation.

The major concern with the use of denosumab is the concern of immune modulation in critical illness. If present, this may be of no consequence, result in benefit through reduction in inflammatory response, or lead to unwanted effects. Although the evidence from antiresorptive trials and bisphosphonate users in critical illness, suggest possible beneficial effects from these classes of agents, we have chosen a conservative approach to administration of denosumab in this study. The intervention will be delayed until infection has been treated (new sepsis or septic shock as defined by Sepsis-3 criteria [86]).

4. HYPOTHESIS AND OBJECTIVES

4.1 Hypothesis: The administration of denosumab to critically ill postmenopausal women will safely and effectively attenuate critical illness associated increase in bone turnover markers.

4.2 Objectives:

- **Primary Objective:** Assess the efficacy and safety of subcutaneous denosumab in postmenopausal intensive care patients requiring longer than 24 hours of mechanical ventilation

- **Secondary Objectives:** Establish whether a phase IIb trial in Australia and New Zealand is justified and feasible, and provide information regarding endpoints necessary in the design of such a trial.

5. STUDY DESIGN AND OUTCOMES

5.1 Design

A prospective, randomised, placebo-controlled, safety and feasibility trial to assess the effects of subcutaneous denosumab on bone mass in post-menopausal female intensive care patients expected to require greater than 24 hours of mechanical ventilation.

5.2 Study population

Inclusion criteria

1. Female
2. Age >50 years or postmenopausal (amenorrhea for greater than 6-months or serum FSH >40mIU/L)
3. Age < 50 years with bilateral salpingo-oopherectomy
4. Expected duration of mechanical ventilation ≥ 24 hrs

Exclusion criteria

1. Unable to undertake BMD (weight >120kg, impaired mobility)
2. Active malignancy
3. Currently receiving immunosuppressive agents
4. Metabolic bone disease
5. Pregnancy
6. eGFR <30ml/min
7. Known contraindication to denosumab (previous reaction, osteonecrosis of the jaw, atypical femoral fracture)
8. Increased risk of osteonecrosis (poor dentition or oral hygiene, dental infection)
9. Hypocalcaemia (<0.9 mm/L ionized calcium)
10. Hypoparathyroidism
11. Malabsorption sydnromes / extensive small bowel resection
12. Neurological condition likely to prevent weight-bearing (eg severe traumatic brain injury, stroke with loss of mobility, degenerative neurological disease)
13. Current treatment with anti-fracture agent (bisphosphonate, denosumab, strontium, teriparatide, within previous 2 years)
14. Current indication for anti-fracture therapy (known BMD T-score < -2.5 and fragility fracture)
15. Treatment limitations in place

5.3 Screening, Enrolment, Randomisation, and Blinding

- Patients in UHG ICU will be screened daily to determine eligibility for enrolment in the trial. If patients fulfill criteria the physician caring for the patient will be approached and asked if they consent to enrolment, after

which the patient or surrogate decision-maker will be approached for consent. A randomisation table and allocation schedule will be created by computer software (i.e. computerised sequence generation) and used by a trials pharmacist at Barwon Health. All personnel, with the exception of the trial pharmacist, will be blinded to treatment allocation. Following patient randomisation the trial pharmacist will dispense the trial drug in a blinded formulation, and the trial drug will then be administered by the ICU bedside nurse, or the trial nurse, according to the study treatment plan.

5.4 Outcome Measures

As this is a safety and feasibility trial the purpose is to establish a treatment effect of denosumab in the study population, and assess potential adverse effects. These results will determine the feasibility of a larger phase II, multi-centre study with change in BMD at 1-year as the primary outcome.

Primary Outcome

- Change in the bone turnover markers collagen type 1 cross-linked c-telopeptide (CTX) and serum type 1 procollagen N-terminal (P1NP) 28-days after administration of study drug.

Secondary Outcomes

- Bone turnover outcomes
    - Change in P1NP, CTX, vitamin D, and PTH from enrolment to 1-year
    - Annualised change in lumbar-spine and femur BMD from enrolment to 1-year
- Safety outcomes
    - Incidence and severity of adverse events (gastrointestinal symptoms, infections, osteonecrosis)
    - Haematogical, biochemical (urea, creatinine, calcium, liver function tests, white cell count, CRP)
- Patient-centred outcomes in the year after ICU
    - Hospital length of stay, level of accommodation
    - Fragility fracture
    - Mortality

Bone mineral density measurement

BMD measurements will occur at 2 separate time-points. The first is between ICU and hospital discharge, the second 1-year post-intervention. BMD will be measured by dual energy x-ray absorptiometry (DXA) (Lunar; GE Healthcare, Madison, Wis, USA), at the proximal femur and lumbar spine. Short-term precision in vivo is 1.6% for the femoral neck and 0.6% for the lumbar spine[1].

Serum bone turnover marker measurement

The serum bone turnover markers collagen type 1 cross-linked c-telopeptide (CTX) and type 1 N-terminal procollagen (P1NP) will be collected at five separate time-points, the day of the first study drug administration, and days 7, 28, 180, and 365 post initial study drug administration. Bone turnover markers will be measured using the automated Roche Modular Analytics E170 analyser. Serum collagen type 1 cross-linked c-telopeptide limit of detection was 10 ng/L with inter-assay coefficient of variations (CVs) of 6.5% at 361 ng/L, 3.8% at 816 ng/L and 3.4% at 3304 ng/L (n = 10). Serum type 1 N-terminal procollagen inter-assay CVs were 4.9% at 73 µg/L, 2.6% at 392 µg/L, and 2.1% at 768 µg/L (n = 10) with a limit of detection of 5 µg/L. Bone turnover markers will be compared to reference ranges derived from an Australian population sample[2].

5.5 Study Treatment Plan

Study plan during ICU admission

Enrolment

Following enrolment baseline demographic and clinical data will be collected, and baseline serum biochemistry, haematology, and biochemical bone turnover marker tests collected. These blood tests will be collected as part of the routine morning blood collection in ICU patients, via existing vascular access (central venous line or intra-arterial line) when present, as is routine practice in ICU. All other ICU care will be carried out as per unit policy and standard practice.

Standard Care

- Standard nutrition will be administered to participants per ICU feeding protocols, including dietician review and advice provided to participants in hospital.
- Vitamin D supplementation:
    - Following enrolment and randomisation, a serum vitamin D level will be collected and analysed. If the serum vitamin D level is < 50 mol/L, a single dose of 50,000 IU cholecalciferol will be administered via oral or enteral route.

Intervention

The intervention to be examined in this trial is the subcutaneous administration of denosumab 60mg compared to placebo (0.9% saline). The first dose of study drug will be given on day 3 in ICU after vitamin D assessment has been completed and supplementation provided, and in the absence of untreated or new infection. The second dose of intervention or placebo will be administered at the 6-month follow-up, after vitamin D assessment and supplementation as indicated.

The first dose of study drug will be administered by an ICU registered nurse as a subcutaneous injection on study day 3 in ICU.

- Placebo:
    - Formulation: 0.9% Saline in a single-use pre-filled 1ml syringe
    - Administration: subcutaneous injection administered in upper arm, upper thigh, or abdomen.
- Denosumab:

- Formulation: 60mg denosumab in a single-use pre-filled 1ml syringe
- Administration: subcutaneous injection administered in upper arm, upper thigh, or abdomen.

- Following administration of the study drug in ICU, monitoring for hypocalcaemia will occur a minimum of twice daily for 48-hours. The majority of patients will have intra-arterial and/or central venous vascular access, with regular blood gas measurement that include calcium performed. If routine testing provides twice-daily calcium additional testing will not be performed. Hypocalcaemia is defined as ionized calcium <0.9 mmol/L, based on ICU protocols for treatment of hypocalcaemia in other settings, ie citrate induced hypocalcaemia with the use of citrate for anticoagulation. Hypocalcaemia will be treated with parenteral calcium, as per hospital dosing and administration protocols, to maintain a target ionized calcium range of 0.9-1.1 mmol/L.

Day 7 and 28 follow-up

- Serum biochemical, haematological, and bone turnover marker testing: At day-7 and 28 participants will be asked to undergo serum biochemistry, haematology, and bone turnover marker tests. Where participants remain as in-patients in UHG or have been transferred to a subacute site, these tests will be collected as part of daily blood tests. Where participants have returned home, participants will be contacted by telephone and asked to undergo testing at their preferred place of pathology testing. Research staff will ensure pathology order forms are made available at the preferred site. Participants with serum vitamin D levels < 50 mol/L, will be offered a single dose of 50,000 IU cholecalciferol via oral or enteral route, either provided at UHG or by their local medical officer.

- Bone mineral density testing: The first BMD assessment will be performed between ICU discharge and day 28. This will be organised to occur either before hospital discharge, or at the day 7 or 28 follow-up, based on participant convenience.

6-month follow-up

- Serum biochemical, haematological, and bone turnover marker testing: At 6-months participants will be asked to undergo serum biochemistry, haematology, and bone turnover marker tests. Where participants remain as in-patients in UHG or have been transferred to a subacute site, these tests will be collected as part of daily blood tests. Where participants have returned home, participants will be contacted by telephone and asked to undergo testing at their preferred place of pathology testing. Research staff will ensure pathology order forms are made available at the preferred site. Participants with serum vitamin D levels < 50 mol/L, will be offered a single dose of 50,000 IU cholecalciferol via oral or enteral route, either provided at UHG or by their local medical officer.

- Study drug: The second dose of study drug will be administered by a registered nurse as a subcutaneous injection at 6-months post-ICU discharge.
    - Placebo:
        - Formulation: 0.9% Saline in a single-use pre-filled 1ml syringe

        - Administration: subcutaneous injection administered in upper arm, upper thigh, or abdomen.
    - Denosumab:
        - Formulation: 60mg denosumab in a single-use pre-filled 1ml syringe
        - Administration: subcutaneous injection administered in upper arm, upper thigh, or abdomen.

1-year follow-up and study completion:

- Serum biochemical, haematological, and bone turnover marker testing: At 1-year participants will be asked to undergo serum biochemistry, haematology, and bone turnover marker tests. Where participants remain as in-patients in UHG or have been transferred to a subacute site, these tests will be collected as part of daily blood tests. Where participants have returned home, participants will be contacted by telephone and asked to undergo testing at their preferred place of pathology testing. Research staff will ensure pathology order forms are made available at the preferred site. Participants with serum vitamin D levels < 50 mol/L, will be offered a single dose of 50,000 IU cholecalciferol via oral or enteral route, either provided at UHG or by their local medical officer.

- Bone mineral density testing: The first BMD assessment will be performed between ICU discharge and 28-day follow-up. This will be organised before hospital discharge, or at the 7 or 28-day follow-up, based on participant convenience. Research staff will accompany participants while they attend the UHG DEXA scan.

- At completion of the study continued treatment with vitamin D and antiresorptive agents will be offered to if an ongoing PBS indication is present. In addition, a letter with results and treatment recommendations will be provided to the participant and copied to their local medical officer.

5.6 Trial Schedule

Softer Study Procedures	
Ventilation duration >24 hours to 7-days duration of mechanical ventilation	
D0-1 ICU Enrolment	Inclusion criteria met, consent obtained
	Baseline and demographic data
	Vitamin D level measured
Day 3 Intervention	Serum PINP, CTX, Vit D, PTH, albumin, calcium, phosphate, urea, creatinine, calcium, liver function tests, white cell count, CRP
	Vitamin D supplement if level <50 nmol/L
	Denosumab 60mg sc vs Placebo administered if no new or untreated sepsis
Day 7 Post-intervention	Serum PINP, CTX, Vit D, PTH, albumin, calcium, phosphate, urea, creatinine, calcium, liver function tests, white cell count, CRP
Day 7-28 Post-intervention	BMD #1
Day 28 Post-intervention	Serum PINP, CTX, Vit D, PTH, albumin, calcium, phosphate, urea, creatinine, calcium, liver function tests, white cell count, CRP
Day 180 Post-intervention	Serum PINP, CTX, Vit D, PTH, albumin, calcium, phosphate, urea, creatinine, calcium, liver function tests, white cell count, CRP
	Vitamin D supplement if level <50 nmol/L
	Denosumab 60mg sc vs Placebo
Day 365 Post-intervention	BMD #2
	Serum PINP, CTX, Vit D, PTH, albumin, calcium, phosphate, urea, creatinine, calcium, liver function tests, white cell count, CRP

Close-out: Vitamin D / calcium / anti-resorptive therapy offered to participants in accordance with guidelines and review by an endocrinologist

5.7 Data collection

Study ID	Enrolment	1st trial drug	7-day	28-day	6-month	1-year
Inclusion / exclusion	+	-	-	-	-	-
Date	+	+	+	+	+	+
DOB	+	-	-	-	-	-
UR	+	-	-	-	-	-
Sex	+	-	-	-	-	-
Level accom	+	+	+	+	+	+
Osteoporosis Risk Factors	+	-	-	-	-	-
Co-morbidity	+	-	-	-	-	-
Medication						
Glucocorticoids	+	+	+	+	+	+
Denosumab	+	-	-	-	+	+
Bisphosphonate	+	-	-	-	+	+
Teriparatide	+	-	-	-	+	+
Strontium Ranelate	+	-	-	-	+	+
Vitamin D	+	+	+	+	+	+
Calcium	+	+	+	+	+	+
Hospital						
Admission date	+	-	-	-	-	-
Discharge date	+	-	-	-	-	-
Discharge status	+	-	-	-	-	-
ICU						
Admission date	+	-	-	-	-	-
Diagnosis	+	-	-	-	-	-
Category	+	-	-	-	-	-
APACHE III	+	-	-	-	-	-
Ventilation duration	+	-	-	-	-	-
CRRT	+	-	-	-	-	-
Nutrition	+	-	-	-	-	-
Discharge date	+	-	-	-	-	-
Discharge status	+	-	-	-	-	-
Biochemistry / haem /BTM						
BMD						
Height	-	-	-	+	-	+
Weight	-	-	-	+	-	+
Dual femur BMD	-	-	-	+	-	+
Dual femur T-score	-	-	-	+	-	+
AP spine BMD	-	-	-	+	-	+
AP spine T-score	-	-	-	+	-	+
Adverse events						
Hypcocalcaemia	-	+	+	+	+	+
Sepsis	-	+	+	+	+	+
Antibiotic duration	-	+	+	+	+	+
New infection	-	+	+	+	+	+
Osteonecrosis	-	+	+	+	+	+
GIT symptoms	-	+	+	+	+	+
Fragility fracture	+	-	-	-	+	+
Status	-	+	+	+	+	+

5.8 Timeline

Time	Event	Status
Jul 15 – Jul 16	Protocol development	Complete
Aug- Oct 16	Funding sourced Safety committee PICF / CTA	
Feb 2017	HREC submission	
Jul 2017	Commence enrolment UHG ICU	
Jun 2018	Complete enrolment	
Jul 2018	Primary outcome complete Initial BMDs complete	
Aug 2018	Results analysis **Primary manuscript preparation** Decision regarding expansion to stage 2 trial	
Dec 2018	Second dose intervention complete	
Jul 2019	Second BMD and BTM complete	

6. SAFETY OF SUBJECTS

As this is a pilot study, adverse events will be monitored throughout the trial by study investigators on a case-by-case basis. All adverse events and serious adverse events related to the trial intervention will be reported to the trial co-ordinating centre. Consistent with other studies in critically ill patients, adverse events already defined and reported as study outcomes will not be reported a second time as serious adverse events. Adverse events and serious adverse events;

- General: Fatigue, headache, musculoskeletal pain, rash
- Electrolyte disturbance: Hypocalcaemia
- Osteonecrosis of the jaw
- New Infections; Skin (erysipelas, cellulititis), abdominal, urinary tract, respiratory, bacteraemia , sepsis or septic shock.

7. DATA MANAGEMENT

Trained staff using a paper source document will collect all data. Data will be entered into a Barwon Health Redcaps database designed by the investigators. Randomised patients will be followed up to death or 12-months post-randomisation (whichever occurs first). Data collection will be restricted primarily to those

variables necessary to define clinical patient characteristics including: baseline demographics, primary diagnoses, physiological parameters, diagnostic interventions, therapeutic interventions and documentation of deaths and other serious adverse events (SAE). Patients and/or their legal surrogate will be asked to provide three possible points of contact (home and close family contact details) to the research staff prior to hospital discharge. Full protocol data will be collected in all patients including those excluded at any stage.

8. SAMPLE SIZE AND STATISTICAL METHODS

Based on the fracture post-ICU and BMD post ICU studies, women aged 55yr and older are at risk of increased bone loss. UHG ICU admitted 6500 women, aged 55 yr and older, between 1998-2016. This represents an annual incidence of 0.1% of the total population. When extrapolated to Australia, this is 23,000 women per annum. Furthermore, emerging evidence suugests that anti resorptive therapy for osteoporosis is associated with a survival benefit. The Boland meta-analysis suggesting that the greatest benefit was among those with a baseline mortality rate of > 10: 1000 p-y, substantially less than the observed mortality rate among ICU survivors of 20% at one year. Within our prospective data, we have not been able to undertake further analysis, to identify a high risk subgroup because of sample size limitations, nor have we been able to identify any female participants aged 55 yr and older who did not experience accelarated bone loss.

The principal aim of this study is to detect the change in the bone resorption marker CTX in participants receiving denosumab compared to those receiving placebo. A prospective RCT conducted in 20 postmenopausal females with chronic critical illness administered 3mg ibandronate intravenously compared to placebo, and followed patients for 14-days. They observed a 34% decrease in serum CTX levels on day 6 compared to a 13% increase in the placebo group. By day 11 there was no difference [78]. A large RCT of denosumab for fracture prevention in women with osteoporosis reported a median decrease of serum CTX of 86% at 1-month compared to placebo [80]. In our prospective study of bone turnover markers and BMD in ICU survivors, we reported a median CTX of 654 [IQR 479–1165 ng/] at baseline, and 315 [162-592 ng/L] at 1-year in female participants, with a population median of 338 ng/L (IQR 212–499) [17].

Given these results we believe a clinically significant effect of denosumab is a 50% reduction in median serum CTX from baseline levels to day 28, compared to no change in the placebo group. A sample size of 7 patients per group will provide a 95% power (2 sided p-value of 0.05) to detect a difference in serum CTX from day 0 to day 28 equal to 2 standard deviations, and an 80% power (2 sided p-value of 0.05) to detect a difference equal to 1.5 standard deviations. With a predicted 20% rate of drop-out or death from enrolment to the 28-day primary outcome time-point, a sample size of 18 participants is required. This figure equates to the anticipated enrollment over a 12-month period at the principal study site.

All data will be assessed for normality. Continuously normally distributed data will be reported as mean (±standard deviation), whereas non-parametric data will be reported using median (interquartile range [IQR]) or frequency distribution. Where normality exists, the primary and secondary outcomes will be analysed

using paired t-tests, with a two-sided p-value of 0.05 considered to be statistically significant. Where changes in outcome are found to be non-symmetrical, Wilcoxon sign rank tests will be employed. Due to small sample size, multivariate analysis will not be performed.

9. ETHICAL CONSIDERATIONS

The observational component of the trial involves collection of bone turnover markers and BMD assessment. Patients will have initial blood tests performed while ventilated and sedated, while subsequent blood tests and both BMD assessments will be performed after participants have regained the ability to consent and understand the implications of enrolment. The interventional aspect of the trial has additional considerations. Firstly, patients with indications for antiresorptive agents will be excluded from the interventional arm and offered treatment according to current guidelines. The remaining population will be asked to participate in the intervention arm of the trial. The use of denosumab or placebo is justifiable as the consequences of accelerated bone loss in a high-risk population of ICU survivors are substantial. This is a study conducted in patients who are unconscious and unable to consent to participation; therefore the patient's legal surrogate will be approached to provide consent for the patient. Patients who recover sufficient cognition to understand the explanation of the study will additionally be asked to consent to continue in the trial. Approval for this protocol will be sought from appropriate regulatory authorities, and from participating hospitals' human research ethics committees.

10. FEASIBILITY

The investigators have a track record in critical care and osteoporosis research, and have conducted the only long-term assessment of bone turnover in survivors of critical illness. We recruited 138 patients, including 69 females, into a prospective observational BMD study over a 4-year period, averaging approximately 16 female participants per year. Given this believe we will achieve enrolment over a 12-month period.

11. FUNDING

Funding for this trial is sought from two funding agent;.

1. **Intensive Care Foundation Research Grant**: In October 2016 the study was successful in an application for **$14,638**
2. **UHG ICU Research Fund:** The UHG ICU will provide additional support for this study from operating budget.

Expenses	Per-patient	Pilot study
Enrolment		18
P1NP,CTx,VitD	$137	$2,466
FSH/LH	$140	$2,520
Week 1		
P1NP,CTx,VitD	$137	$2,466
Denosumab	$200	$1,800
ICU discharge/ day 7		
BMD1	$136	$2,448
1-month		
P1NP,CTx,VitD	$137	$1,918
6-months		
P1NP,CTx,VitD	$137	$1,370
Denosumab	$200	$1,800
1-year		10
P1NP,CTx,VitD	$137	$1,370
BMD2	$136	$1,360
Research Co-ord		
8 hrs per patient	$320	$5,760
Statistics	-	-
Pharmacy	-	-
Meetings/support		-
Total	**$2,517**	**$25,528**
Income		
ICF grant		$14,638
ICU research fund		$10,890
Total		**$25,528**

*Assumes 20% dropout/death from enrolment to 28-day primary outcome measure.

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