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Socs3 is a target of oncostatin M in osteocytes that is required for normal bone formation

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Oncostatin M (OSM) is an IL-6 family member that stimulates osteoblast differentiation and RANKL expression. OSM signals by an OSM receptor (OSMR):glycoprotein 130 (gp130) heterodimer, and was shown recently to also signal via a leukemia inhibitory factor (LIF) receptor (LIFR):gp130 heterodimer. In mice, this OSM:LIFR:gp130 pathway specifically activates bone formation by reducing osteocytic sclerostin without upregulating RANKL - a potential therapeutic pathway for osteoporosis.

Downstream targets of OSM:LIFR were determined by microarray expression profiling of OSM-treated wildtype (intact OSM:OSMR and OSM:LIFR) and OSMR null (OSM:LIFR only) primary osteocyte-like cells (calvarial osteoblasts differentiated until osteocytic genes were expressed). Mice with osteocyte-specific deletion of the most highly regulated gene were then examined.

OSM:LIFR signalling targets identified in OSMR null cells comprised a subset of the genes regulated by OSM in wildtype cells. Socs3 (Suppressor of Cytokine Signalling 3), an inhibitor of STAT3 and STAT1, showed a proportionately greater increase in response to OSM in OSMR null cells vs wildtype. OSM stimulation of Socs3 was verified by qPCR of independent cell preparations. STAT3 phosphorylation at Y705 and S727 was rapidly stimulated by OSM treatment of OSMR null cells, consistent with STAT3-dependent stimulation of Socs3.

To determine the role of osteocytic SOCS3 in bone formation, we generated mice lacking SOCS3 specifically in osteocytes (DMP1Cre.SOCS3f/f). 12-week-old male femora were analysed by microCT (n≥10/group). DMP1Cre.SOCS3f/f mice had dramatically lower (~50%, p<0.05) trabecular bone volume and trabecular number (p<0.01). Preliminary histology indicated more osteoid, osteoblasts and osteoclasts in DMP1Cre.Socs3f/f sections.

These data indicate that SOCS3 within the osteocyte restrains both bone formation and resorption by suppressing STAT3 and STAT1. The similarity in phenotype of the DMP1Cre.SOCS3f/f mice with an earlier model with global STAT1/STAT3 hyperactivity (gp130757F) suggests that high bone turnover resulting from systemic STAT1/STAT3 activation can be reproduced by targeting activation to the osteocyte alone.
SQSTM1/p62 mutant proteins associated with Paget’s disease of bone lead to increased autophagy markers, while attenuating autophagosome maturation.

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Background: Mutations in the SQSTM1/p62 gene are associated with Paget’s disease of bone. The SQSTM1/p62 protein is considered to be a signalling hub linking NF-κB signalling, oxidative stress signalling (Keap1/Nrf2), autophagy and apoptosis. We have previously shown that over-expression of certain mutant SQSTM1/p62 proteins leads to hyperactivation of NF-κB and increased osteoclastogenesis, but the underlying mechanisms are largely unclear.

Aims: This study aimed to determine the affects of PDB-associated mutations on the function of p62 in autophagy processes, including autophagosome maturation.

Methods: We have used luciferase assays to determine the effect of 7 mutant p62 proteins on NF-κB activation in non-stimulated cells. We conducted co-immunoprecipitation experiments, where HEK293 cells were transfected with expression plasmids for FLAG-p62 (wild type or mutant) or empty vector. p62 proteins were immuno-precipitated with FLAG antibody and the bound LC3 detected by Western blot analysis. Additionally, we have used a previously validated system to determine the effect of these mutations on autophagosome maturation into protein degrading autophagolysosomes; we transfected a cell line stably expressing mCherry-GFP-LC3 with p62 expression constructs and using confocal microscopy determined the ratio of autophagosomes and autophagolysosomes formed in cells expressing mutant or wild type p62.

Results and Conclusions: Our data shows that SQSTM1/p62 mutant protein expression correlates significantly increased NF-κB activity with increased physical interaction between p62 and the autophagy marker LC3. However, mutant proteins also attenuate autophagosome maturation. By contrast, expression of wild type SQSTM1/p62 promotes autophagosome maturation. Together, our data suggests that SQSTM1/p62 mutant proteins are defective mediators of the final stages of autophagy and this may be important for NF-κB regulation via perturbed degradation of specific protein substrates.
SNORD116 deficient mice as a skeletal model of Prader-Willi Syndrome

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Prader-Willi Syndrome (PWS), a leading cause of genetic obesity, is a disorder characterised by insatiable appetite, poor muscle development, cognitive impairment, hormonal imbalance, short stature and osteoporosis. A number of causative loci have been postulated for PW, all located in the Prader-Willi Critical Region (PWCR) located on chromosome 15(q11-13). This particular region of chromosome 15 is maternally imprinted, and PWS individuals display dysfunction and or deletion of the non-silenced paternal chromosome.

SNORD116 produces a small non-translated nucleolar RNA (snoRNA) which resides within the PWCR as a 29 copy cluster of unknown function. SNORD116 is largely expressed in the brain in humans and exclusively brain expressed in mice. Loss of SNORD116 has recently been associated with the PW phenotype, providing a platform for further investigation in mice.

This study utilised SNORD116 knockout mice in an attempt to ascertain how SNORD116 affects the skeleton. Consistent with PWS, SNORD116 KO mice showed impaired skeletal development as determined by shorter femurs. DXA, µCT and bone histomorphometric analysis, revealed a reduction in bone mass due to deficiency in cortical bone volume but no change in cancellous bone. Interestingly, cortical mineral apposition rate was lower in KO mice. However, SNORD116 KO mice do not exhibit all the symptoms of PWS.

SNORD116 deletion in mice recapitulates the short stature and low BMD of PWS individuals. SNORD116-deficient mice may be a suitable model for the skeletal effects in PWS and demonstrates a novel central pathway in cortical bone homeostasis, suggesting that non-translated RNA, expressed solely within the brain can regulate cortical bone mass.
Endochondral ossification and advanced enthesitis are key features of PGISp mouse model of ankylosing spondylitis

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Ankylosing spondylitis (AS) is an inflammatory arthritis specifically targeting the spine and pelvis in which inflammation leads to osteoproliferation around and ultimately across affected joints, resulting in joint fusion (ankylosis). No therapies are currently available that prevent or even slow this inevitable progression that results in significant disability.

AS has two phases, an initial inflammatory phase similar to other inflammatory arthritides with high levels of pro-inflammatory cytokine production, followed by an osteoproliferative/ankylosing phase which causes the bulk of the disability. Very little is known about the progression from the inflammatory stage to the pathologic bone formation.

The proteoglycan-induced mouse model of spondylitis (PGISp) presents with many similarities with human AS with extensive inflammation-induced osteoproliferation occurring in the spine and pelvis. We have characterised the molecular and tissue changes occurring during disease progression to joint fusion over a six-month time course.

Disease commences with mild inflammation at the periphery of the intervertebral disc (IVD) which progresses invading and destroying the IVD driven by elevated MMP-3, MMP-13 and TNF-α mRNA expression. Inflammation then resolves accompanied by mesenchymal cell expansion and chondrocyte formation appearing at the periphery of the IVD resulting in huge amounts of excessive tissue laid down. Similar to human disease ectopic chondrocyte expansion was evident adjacent to the longitudinal ligaments. The excess tissue was mainly composed of a mineralised type II and X collagen positive cartilaginous matrix. A number of pathways were perturbed including the Wnt pathway which might regulate both cartilage degradation and bone formation in a biphasic fashion. Osteoclasts also appeared to be reactivated in the later timepoints suggesting the cartilaginous matrix might be remodelled progressing to bone.

Thus it appears endochondral ossification is underlying the excessive tissue formation in this model and suggests new therapeutic avenues targeting the endochondral ossification process might be viable.
Abstract for Garvin Institute Symposium November 2013

The Role of N-Cadherin in Multiple Myeloma

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Introduction: Multiple myeloma (MM) is an incurable B-cell malignancy characterised by the clonal proliferation of malignant plasma cells (PCs) within the bone marrow (BM). Expression of CDH2, the gene encoding for the cell-adhesion molecule N-cadherin, is elevated in CD138⁺ MM PCs from approximately 50% of newly diagnosed MM patients (Groen et al. 2011; Vandyke et al. 2013). In addition, serum N-cadherin levels are elevated in a subset of MM patients who have high-risk disease and poor prognosis (Vandyke et al. 2013).

Aim: To investigate the role of N-cadherin in the modulation of MM PC behaviour in MM tumour establishment and progression.

Methods: PC proliferation and adhesion assays and assessment of tumour burden in vivo was performed using the Xenogen IVIS Imaging System.

Results: Using the mouse myeloma 5TGM1 PC line, our studies showed that shRNA-mediated N-cadherin knock-down (k/d) significantly reduced PC attachment to both BM stromal cells (BMSCs) and BM endothelial cells (BMECs) suggesting that N-cadherin is an important mediator of PC-microenvironment interactions. In the context of cell proliferation, 5TGM1-N-cadherin k/d PCs displayed a ~30% reduction in proliferative capacity compared with control cells after 3 days. Using the C57BL/6 KalwRijHsd mouse model, our studies showed that mice injected with 5TGM1 N-cadherin shRNA k/d PCs had significantly reduced tumour burden after 4 weeks compared with mice injected with control 5TGM1 scramble shRNA PCs. Notably, daily intraperitoneal administration of the N-cadherin antagonist ADH-1 (Exherin™; 50mg/kg/day) to 5TGM1 PC-injected mice significantly decreased tumour burden after 4 weeks, when compared with mice treated with PBS vehicle alone.

Conclusion: These studies highlight the possibility that targeting N-cadherin may provide a novel therapeutic strategy to control MM disease progression in patients with aggressive disease.

References


Myeloma plasma cells alter the bone marrow microenvironment by stimulating an increase in mesenchymal stromal cells

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Background: Multiple myeloma (MM) is an incurable haematological malignancy characterised by the clonal proliferation of plasma cells within the bone marrow (BM). Numerous studies suggest that the myeloma plasma cells occupy and alter the stromal tissue of the BM as a means of enhancing their survival and growth. However, the nature and magnitude of the changes to the stromal cell tissue remain to be determined.

Aims: To identify changes in the stromal tissue in response to myeloma plasma cells.

Methods: In this study, we utilised mesenchymal stromal cell (MSC) and osteoblast (OB)-related cell surface marker expression and flow cytometry to enumerate MSC and OB numbers in diagnostic BM recovered from myeloma patients and C57BL/KaLwRij mice bearing myeloma disease.

Results: Using this approach, we identified an increase in the number of STRO-1 positive colony forming MSC and a concomitant decrease in alkaline phosphatase positive OB. Notably, this increase in MSC numbers correlated closely with plasma cell burden at the time of diagnosis. Additionally, in comparison with the OB population, the STRO-1+ MSC population was found to express higher levels of plasma cell- and osteoclast- activating factors, including RANKL, CXCL12 and IL-6, providing a mechanism by which an increase in MSC may promote and aid the progression of myeloma. Importantly, these findings were faithfully replicated in the C57BL/KaLwRij murine model of myeloma.

Conclusion: Myeloma plasma cells alter the bone microenvironment by stimulating an increase in mesenchymal stem cells. In addition, the C57BL/KaLwRij murine model presents a clinically relevant system in which to identify and therapeutically modulate the bone microenvironment and in turn, alter the progression of myeloma disease.
Interleukin-33 Stimulates Functional Osteoclast Formation in the Absence of RANKL

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Formation of active osteoclasts depends critically on RANKL, evident by the lack of osteoclasts in RANKL null mice. However, several cytokines including TNF and LIGHT (a TNF-related protein) may drive human osteoclast formation in human monocytes independently of RANKL. IL-33 is an IL-1 family member and Th2 stimulating cytokine produced by osteoblasts, and Mun et al. (2011, Cell Mol Life Sci 67: 3883) found IL-33 caused TRAP⁺ multinucleated cell (MNC) formation from monocytes. However, this was inconclusive regarding osteoclastogenesis since their ability to resorb bone or dentine was not examined. Two later studies showed no osteoclastogenic response to IL-33. We therefore attempted to clarify IL-33 actions.

We examined osteoclast formation in adult monocytes and GM-CSF-expanded human cord blood mononuclear cells (CBMC) in long term culture on dentine substrate, treated by either RANKL or IL-33 plus M-CSF, an essential proliferation co-factor. Formation of TRAP⁺ MNCs and resorption pits were examined. We also studied effects of RANKL and IL-33 on murine bone marrow macrophages (BMM) and RAW264.7 cells.

RANKL-treated adult monocytes formed many large bone resorbing TRAP⁺ MNCs. This also occurred with IL-33 treatment but only in a minority of blood samples, 3 of 12 individuals tested. Expanded CBMCs formed bone resorbing osteoclasts only with RANKL, not IL-33. Similarly, IL-33 treatment did not elicit TRAP⁺ MNC formation from BMM and RAW264.7 cells. Indeed, IL-33 elicited NFκB signals but did not increase NFATc1 or MITF-E mRNA levels. However, TRAP⁺ mononuclear cells were noted in IL-33 treated RAW264.7 cultures, suggesting some weak response. Addition TGFβ (to boost osteoclastic responsiveness) to IL-33-treated RAW264.7 cells caused a few (8±1/well) osteoclasts to form, but far less than RANKL treatment (183±3/well).

Thus, IL-33 does indeed display osteoclastogenic effects, which may have pathophysiological significance. However, the weak, inconsistent nature of this stimulus may explain conflicting reports about IL-33.
The physiological context of bone loss in advanced cancer.

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Alongside the challenges of treating primary and metastatic tumours, advanced cancer patients suffer a range of conditions that reduce quality of life and also contribute to metastasis and poor survival. This cluster of syndromes includes the fatigue and muscle wasting of cancer cachexia, propensity for blood clots, immune system defects, chronic pain as well as hypercalcemia of malignancy. Mobilisation of calcium from bone is a prominent feature of diverse cancers such as breast, lung, renal and multiple myeloma. Hypercalcemia can result from activation of osteoclast resorption and altered renal reabsorption by kidneys due to release of factors from tumours such as PTHrP. In addition, the recent appreciation of the critical role of gp130-mediated signalling in maintenance of bone integrity highlights the potential of other tumour-derived factors such as IL-6 family cytokines in bone defects associated with cancer. As 25% of all cancers arise in an inflammatory setting with many tumours dependent on IL-6 and IL-11 production by malignant and/or normal cells within the tumour for growth and progression, it is likely that the spill-over effects of cytokine release into circulation disrupts the finely balanced gp130 signalling in bone and other organ systems required for metabolic homeostasis.

The murine colon C26 carcinoma is a well-characterised model of both hypercalcemia and cancer cachexia that are apparently driven by tumour-derived PTHrP and IL-6 respectively. We have explored dysregulated metabolic pathways in organs involved in cachexia including skeletal muscle, heart, liver, white and brown adipose tissue. This provides an integrated physiological framework for understanding the interplay of signalling pathways and the cumulative metabolic defects operative in multiple organs within which bone loss occurs in cancer. One potential link is the impact of inflammatory MAPK-mediated signals downstream of the IL-6 receptor on type II nuclear receptors due to impaired RXR action.
**Cell communication in osteoimmunology**

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RANKL is a key cytokine for osteoclast differentiation expressed by mesenchymal cells including osteoblastic cells as well as T cells, thus it has been difficult to precisely characterize RANKL-expressing cells under physiological and pathological settings (1, 2). The source of RANKL in bone has been thought to be osteoblasts or bone marrow stromal cells, but osteocyte-specific RANKL knockout mice revealed the crucial role of osteocytes in supporting osteoclastogenesis in adult bone remodeling (3). RANKL signal transduction is mediated by numerous immune-related molecules including NFATc1 and ITAM, which shed light on the mechanisms shared by the bone and the immune systems (2).

Thus, the immune and skeletal systems share various molecules, including cytokines, transcription factors, signaling molecules and membrane receptors. Here I will discuss the emerging topics in osteoimmunology including bone cell communication factors in the regulation of bone formation by osteoclast-derived Sema4D (4) and the osteoprotection by Sema3A (5).

**In vivo monitoring of bone and fat in mice using the Quantum Fx scanner**

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Monitoring of bone mass and fat mass *in vivo* is usually performed using dual energy X-ray absorptiometry (DXA), providing quantification of fat and lean mass and the determination of BMD and BMC. DXA is 2-dimensional and therefore, volumetric bone data, as well as differentiation of visceral and subcutaneous fat is not possible.

Currently, we have been piloting the use of an *in vivo* 3-dimensional microCT, the Quantum Fx. This technology scans down to 10\(\mu\)m, and generates high-quality CT images which can be segmented and volumes rendered based on tissue density. The Quantum Fx has several advantages: short scan time and low X-ray doses, thus scans can be performed more frequently and applied to longitudinal studies.

Like existing microCT, the Quantum Fx produces consistent scans of skeletal tissue; however we sought to examine its ability to track changes in adipose tissue. We have analysed a set of mice over time to determine its ability to monitor changes in fat volume. The total fat volumes generated from the scan correlated with DXA data from the same mice. By further segmentation of the fat into abdominal subcutaneous and visceral fat, we were able to track the deposition of fat over time. In addition to the *in vivo* capabilities of the Quantum Fx, excised bones can be scanned at high resolution for cortical and cancellous analysis.

Based upon our initial observations, the Quantum Fx is capable of simultaneous monitoring of both bone and adipose tissue in longitudinal studies.
Prediction of fracture and fracture-associated outcomes

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Background and Aim — Existing predictive models in osteoporosis were developed to predict the risk of an initial fracture only. This study was aimed at developing a model for predicting the risks of initial fracture, subsequent fracture, and mortality.

Methods — The study involved 757 men and 1224 women, aged 60 years and older, whose health outcomes had been monitored for up to 24 years. Fracture was ascertained using X-ray report. Femoral neck bone mineral density (BMD) was measured by DXA. A multistate Markov model was used to estimate the transition probabilities between three events (ie initial fracture, refracture, and mortality).

Results — Among women without a fracture, approximately 37% would remain fracture-free during the subsequent 10-year follow-up; 24% would suffer a fracture, and 8% went on to refracture, and another 32% would die during the period. Among women with an initial fracture, 56% died within 10 years, and 60% died after a refracture. Among men without a fracture, 38% would remain fracture-free, 14% would sustain a fracture, and 45% would die during the next 10 years. Among men with an initial fracture, the risk of a refracture was 16%, and the mortality risk was 76%. In both genders, the advancing age and reduced BMD were main predictors of the 3 events.

Conclusions — Individuals with an initial fracture have an increased risks of subsequent fracture and mortality. The strong effects of age and BMD on the three related events allow an individualized approach to the risk assessment of fracture and its associated outcomes.
Calcium plus Vitamin D supplementation: a Meta-analysis of Risk and Benefit

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Background and Aim — We sought to examine the effects of calcium and vitamin D (CaD) supplementation on fracture risk and cardiovascular disease (CVD) outcomes.

Methods — We identified 9 primary RCTs on the efficacy of CaD on fracture risk, and 3 post-hoc analyses of RCT on the association between CaD and CVD outcomes. The 9 RCTs on fracture involved 27705 patients on CaD and 25491 patients on placebo. The 3 RCTs with CVD outcomes involved 10081 individuals on CaD and 9909 on placebo. The data were synthesized by the Bayesian random-effects meta-analysis.

Results — CaD supplements reduced the risk of fragility fracture by 11% (RR 0.89; 95%CI 0.80-0.97), and the reduction was observed in nonvertebral fractures (RR 0.90; 0.79-0.99) and clinical vertebral fracture (RR 0.86; 0.75-0.99), but not in hip fracture (RR 0.88; 0.71-1.06). Meta-regression analysis suggested that the anti-fracture benefit of CaD supplements was more likely observed in individuals with 2% and greater risk of fracture per year. CaD supplements were not significantly associated with any CVD outcome: myocardial infarction (RR 1.18; 0.73-1.74), stroke (RR 1.17; 0.75-1.70), myocardial infarction or stroke (RR 1.14; 0.74-1.64), and death (RR 1.01; 0.67-1.58). The number needed to treat to reduce a fracture was 85, and the number needed to incur a CVD event was 170. Thus, the ratio of benefit over potential risk was 2.

Conclusions — CaD supplements reduce the risk of fracture, but the effect size is likely modest. The association between CaD supplements and CVD outcomes is uncertain.

Risk ratio of fracture and CVD outcomes associated with CaD supplements

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Relative Risk (RR) and 95% CI</th>
<th>Probability of RR&lt;0.90 (for fracture) or RR&gt;1.10 (for CVD outcomes)</th>
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<tbody>
<tr>
<td>All fractures</td>
<td>0.89 (0.80 – 0.97)</td>
<td>0.48</td>
</tr>
<tr>
<td>Vertebral fracture</td>
<td>0.86 (0.75 – 0.99)</td>
<td>0.59</td>
</tr>
<tr>
<td>Non-vertebral fractures</td>
<td>0.90 (0.79 – 0.99)</td>
<td>0.46</td>
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<tr>
<td>Hip fracture</td>
<td>0.88 (0.71 – 1.06)</td>
<td>0.47</td>
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<tr>
<td>Myocardial infarction (MI)</td>
<td>1.18 (0.73 – 1.74)</td>
<td>0.63</td>
</tr>
<tr>
<td>Stroke</td>
<td>1.17 (0.75 – 1.70)</td>
<td>0.62</td>
</tr>
<tr>
<td>MI or stroke</td>
<td>1.14 (0.74 – 1.64)</td>
<td>0.59</td>
</tr>
<tr>
<td>Death</td>
<td>1.01 (0.67 – 1.58)</td>
<td>0.30</td>
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MULTIPLE MEASUREMENTS OF QUADRICEPS WEAKNESS PREDICT FRACTURE RISK

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The association between muscle strength and fracture is not well understood. The present study was aimed at examining the contribution of baseline and serial measurements of Quadriceps Strength (QS) to risk of fragility fracture in elderly.

The study involved 595 men and 1065 women aged 60+ years (median: 69) followed for a median of 11 years. The median follow-up time from the last QS measurement was three years. At baseline and approximately two-year intervals, QS was measured by a dynamometer. Only individuals with at least three QS measurements were included in the analysis. Femoral neck BMD was measured by dual energy X-ray absorptiometry (GE- Lunar). Low-trauma fracture was ascertained from X-ray reports. The magnitude of association between QS and fracture was assessed by time-variant Cox’s regression analysis.

During the follow-up period, 89 (14.96%) men and 281 (26.38%) women sustained fragility fracture. In time-variant analysis, each 10 kg decrease in QS was associated with 61% increased risk of fracture in men (hazard ratio [HR] (95% CI): 1.61 (1.29 to 2.02)) and 37% increased risk in women (HR: 1.37 (1.16 to 1.62)). After adjusting for FNBMD, age at baseline, fracture prior to baseline and history of falls, the hazard ratios (95% CI) were 1.47 (1.16 to 1.85) for men and 1.21 (1.02 to 1.44) for women.

In time-fixed analysis, baseline QS was associated with increased risk of fracture in men (adjusted HR (95% CI): 1.61 (1.25 to 2.08)). In women, baseline QS was significantly associated with increased risk of fracture in univariate analysis (HR (95% CI): 1.25 (1.07 to 1.46)), but not in multivariate analysis.

These data indicate that quadriceps weakness is an independent determinant of fracture risk. The accuracy of fracture risk prediction could be improved by the incorporation of serial measurements of quadriceps strength.
Suggestive loci for osteoporosis: a multipoint variance component linkage analysis of extended pedigrees

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Background and Aim – Genome-wide linkage analysis using extended pedigrees is an attractive approach to search for genes for complex diseases. The primary aim of this study was to identify loci linked to bone mineral density (BMD) and quantitative ultrasound measurements (QUS).

Materials and Methods – The Dubbo Osteoporosis Genetics Study is a large multi-generational family study involving 141 pedigrees. Individuals are recruited through probands with high BMD (Z-score > +1.28). BMD (g/cm$^2$) at the femoral neck, lumbar spine, and whole body were measured by DXA (GE-LUNAR). QUS measurements were obtained by a Sunlight Omnisense. Subjects were genotyped using 530 microsatellite markers. After adjusting for age, sex and other covariates, we performed a variance components linkage analysis using SOLAR.

Results – We analysed 1007 individuals (636 women) aged 58 ± 19 (mean ± SD; range 18-98). We found suggestive linkage for BMD at the whole body (LOD score 2.02, chromosome 6) and for QUS at the tibia (LOD score 2.77, chromosome 18), but not for femoral neck (highest LOD 1.50, chromosome 6) or lumbar spine (highest LOD 1.38, chromosome 12). We also stratified by age and found suggestive linkage for femoral neck and lumbar spine BMD both on chromosome 12 (LOD scores 2.88 and 1.82, respectively) for individuals aged 50-80 years.

Conclusions – These data suggest the presence of multiple loci regulating bone mineral density and quantitative ultrasound measurements. The identification of specific markers or genes may lead to better prediction of fracture risk and individualised osteoporosis treatment.
Genetic contributions to bone mineral density and quantitative ultrasound measurement: an analysis based on exact identity-by-descent sharing within pedigrees
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Background and Aim – The estimation of genetic contribution to skeletal traits has largely been based on twin and family studies which are based on strong assumptions regarding genetic sharing and environmental effects. In this study, we sought to estimate the degree of genetic contribution to the variation in bone mineral density (BMD) and quantitative ultrasound measurements (QUS) by using the assumption-free identity-by-descent sharing between siblings.

Materials and Methods – The study involved 352 men and 606 women from 436 nuclear families, including 138 extended pedigrees of Caucasian background. BMD at the femoral neck, lumbar spine, and whole body was measured by dual energy X-ray absorptiometry (GE-Lunar, Madison, MI). Ultrasonography at the forearm (distal radius), tibia (midshaft), and digits (phalanges) was measured by Sunlight Omnisense (BeamMed, Petach-Tikva, Israel). Using a variance components method and the exact identity-by-descent sharing between relatives, we estimated the variance due to additive genetic factors, common and specific environmental factors, after adjusting for covariates.

Results – The average (SD) age of the study subjects was 56.9 (19.1) for men and 57.7 (17.3) years for women. After adjusting for age, gender, and body weight, genetic factors explained 45%, 56%, and 60% of residual variance in femoral neck, lumbar spine, and whole body BMD, respectively. No significant effect of common environmental variance was found in any BMD site. Genetic factors also accounted for 35%, 40%, and 21% of residual variance in QUS at the forearm, tibia, and digits, respectively. Significant shared environmental factors were observed at the forearm and digits.

Conclusions – These data indicate that approximately half of the residual variation in bone density is attributable to genetic factors. However, the degree of genetic effect on quantitative ultrasound parameters is modest. These findings underline the importance of genetic factors in the pathogenesis of osteoporosis.
Using 3D bioluminescence and µCT to monitor tumour progression and bone metastasis in breast and prostate murine models

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Monitoring tumour metastases in vivo is commonly performed using two dimensional (2D) bioluminescent imaging (BLI). 2D BLI involves quantification of light-emitting from an animal surface generating a relative measure of internal light source brightness. As this 2D method does not take into account source depth to obtain absolute source brightness, accurate localisation of metastatic tumours is limited. Recent advances have led to the development of 3D in vivo BLI, however, it is unknown whether these imaging systems have the ability to detect metastatic lesions localised to the skeleton. We aimed to examine the potential for 3D in vivo BLI to accurately localise skeletal metastases through co-registration with micro computerised tomography (µCT), using the IVIS Spectrum and Quantum Fx imaging systems. Following intra-cardiac inoculation of MDA-231LUC2 or PC3LUC2 human cancer cells into Balb/Cnu mice, metastatic bone disease developed, as previously reported. 3D BLI utilising the IVIS Spectrum demonstrated growing tumours at sites associated with skeletal structures. Co-registration of these BLI images with Quantum Fx µCT scans allowed for precise 3D localisation of these tumours within the skeleton. Ex vivo analyses with high resolution µCT and histology confirmed skeletal tumour localisation. Based on these findings, the IVIS Spectrum and Quantum Fx together, accurately detects and localises skeletal metastases in human MDA-231LUC2 and PC3LUC2 models through 3D in vivo bioluminescent imaging. This novel 3D imaging technique will become an invaluable tool for monitoring tumour growth in skeletal tissue in longitudinal studies.
Visualization Of Tumor Cell Dormancy And Activation In The Skeleton By Two-Photon, Intra-Vital Imaging

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Cancer cells arrive in the skeleton where they exist in a dormant state or are activated to form tumours. Our understanding of these events is limited due to an inability to visualize in vivo in bone. Using multiple myeloma as a model, we developed novel labelling and intra-vital imaging techniques to visualize the arrival of tumour cells in bone, locate individual dormant cells and examine their activation in live mice.

5TGM1eGFP murine myeloma cells were injected (i.v.) into C57BLKalwRij mice during intra-vital (live) 2-photon microscopy to examine their arrival in the bone marrow of intact tibia. 5TGM1eGFP cells were labelled with a membrane dye (Vibryant DiD), which is retained by dormant cells (DiD High), but lost on sharing through division (DiD Neg). Cells were also injected and visualized after 1, 7, 14, 21, or 28 days, in live mice by intra-vital 2-photon microscopy. DiD High and DiD Neg cells were isolated for flow cytometry, CD138+ve myeloma cells and colonies were identified by immunohistochemistry.

Intra-vital microscopy revealed that tumour cells in bone can both circulate through the vasculature or colonise, actively migrating towards bone surfaces. Upon colonisation, individual, DiD High, cells were detected directly opposed to endosteal bone surfaces which was confirmed by flow cytometry (171±31/10^6 total cells) and CD138+ve immunohistochemistry. DiD Neg/GFP+ve cells could be identified from day 14, which increased through to day 28. This was associated with formation of a limited number of CD138+ve colonies at day 21 (14.8±1.1). Individual DiD High cells remained evident at day 28.

These data demonstrate that two-photon, intra-vital microscopy can be used to visualize the arrival of tumour cells in bone, confirming a limited number colonise and survive here. Further, this technique can be used to localise dormant cells and demonstrate their activation in bone. We also show that only a limited number of dormant cells in bone are activated to form tumour colonies.
ISOLATION AND TRANSCRIPTOME ANALYSIS OF DORMANT MYELOMA CELLS
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Myeloma is a malignancy with affinity to bone, which provides permissive microenvironment for myeloma survival and growth. Myeloma cells can exist there in a dormant state or, upon receiving activation signals, proliferate and form tumours. As dormant myeloma cells have not been phenotypically and functionally characterized yet, our aim was to isolate and assess transcriptome profile of dormant myeloma cells, and provide insight into signals involved in transition from dormancy to proliferative state.

5TGM1eGFP myeloma cells were labeled with a fluorescent membrane dye (DiD), which is, upon division, distributed among daughter (DiD<sub>low</sub>) cells, and eventually lost after certain number of divisions (DiD<sub>neg</sub> cells). Non-dividing (DiD<sub>high</sub>) cells, retain high level of fluorescence. DiD labeled cells were injected (i.v.) into C57BLKAlwRij mice and their frequency was assessed 7, 14, 21, or 28 days post-injection by flow cytometry. DiD<sub>high/low/neg</sub> populations were sorted by FACS, their total RNA was extracted and cDNA hybridized on mouse ST 2.0 whole genome arrays (Affymetrix).

Flow cytometry detected DiD<sub>high</sub> cells in the bone marrow through all time points. DiD<sub>neg/GFP+ve</sub> cells appeared on day 14, and increased to day 28. Differential gene expression analysis identified 250 differentially expressed genes in DiD<sub>high</sub> vs. DiD<sub>neg</sub> cells, and absence of differentially expressed genes between DiD<sub>low</sub> and DiD<sub>neg</sub> cells (Q value < 0.05, FC > 2.0). Functional annotation tools and gene set enrichment analysis (GSEA) identified immune system-related pathways as important determinants of quiescence, in particular interferon (IFN) type I, cytokine and integrin signaling. In addition GSEA confirmed the quiescent character of DiD<sub>high</sub> cells with down-regulation of gene sets involved in proliferation, cell cycle progression and division.

This confirmed the long-term presence of dormant myeloma cells during the course of disease, which have a unique transcriptome profile, and identified potential candidate genes critical in transition to and retaining dormancy.
Developing a Biphasic Scaffold for Osteochondral Regeneration

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Introduction & Aim: Damage and degeneration of osteochondral tissue at skeletal joints are very difficult to repair, particularly for full-thickness osteochondral defects involving both articular cartilage and subchondral bone. Existing clinical treatments are aimed at alleviating pain and morbidity in the short term, while long term treatment is rarely successful and often leads to the progression of osteoarthritis. Biomaterials-based osteochondral tissue engineering is emerging as a novel treatment strategy that can address the growing unmet clinical need to develop more effective therapies. The aim of the present study was to develop a biomaterial scaffold targeted at the effective treatment of full-thickness osteochondral defects.

Methods: A novel biphasic scaffold was developed in this study, with two different phases respectively targeted at the regeneration of cartilage and subchondral bone in full-thickness osteochondral defects (Fig. 1). The biphasic scaffold was constructed by integrating a flexible and resilient silk scaffold which was the cartilage phase, with a mechanically strong and bioactive composite scaffold (silk-coated ceramic scaffold) which was the bone phase. Physical, mechanical and biological properties of the biphasic scaffold were determined with respect to application in osteochondral regeneration.

Results: Scanning electron microscopy showed that the two phases of the biphasic scaffold imitated the structural characteristics of native cartilage and bone. Mechanical testing showed that the biphasic scaffold was mechanically competent and suitable for implantation in load-bearing osteochondral defects. \textit{In vitro} testing showed that the biphasic scaffold supported the growth of human mesenchymal stem cells and could induce tissue-specific differentiation responses in the two phases, indicating its ability to encourage the respective regeneration of cartilage and bone.

Conclusion: The developed biphasic scaffold has potential for future clinical application in the reconstruction of osteochondral defects that will block disease progression to osteoarthritis.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{biphasic_scaffold.png}
\caption{Biphasic scaffold with two different phases respectively targeted at cartilage and bone regeneration in full-thickness osteochondral defects.}
\end{figure}
Prolonged Exposure Is Required For Low Concentrations Of Bisphosphonate Drugs To Inhibit Protein Prenylation

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Bisphosphonate drugs (BPs) target bone, are selectively internalised by osteoclasts at high (at least mM) concentrations during bone resorption, and prevent the prenylation of small GTPases such as Rap1A. We sought to determine whether low, nM concentrations of the BP zoledronic acid (ZOL), ie concentrations might occur outside the skeleton, could inhibit protein prenylation in cultured macrophages. J774 mouse macrophage cells were cultured acutely (1-2 days, mimicking effects of BP on osteoclasts) or chronically (up to 31 days, mimicking possible effects of circulating BP on cell types outside the skeleton) with 1mM-50mM or 1nM-1000nM ZOL. The accumulation of unprenylated Rap1A (which reflects the inhibition of protein prenylation by ZOL) was assayed via western blot analysis of cell lysates. Acute treatment with >2mM ZOL inhibited Rap1A prenylation, but concentrations <1mM had no detectable effect. In contrast, treatment for 7 days with 1mM ZOL dramatically inhibited Rap1A prenylation treatment but was without effect after 2 days. By day 31, prolonged exposure to as low as 10 nM ZOL markedly inhibited prenylation. We provide the first evidence that nM concentrations of the BP drug ZOL can inhibit protein prenylation in cultured macrophages, but that prolonged exposure to low concentrations of ZOL is required (as might occur in vivo in patients on long-term BP therapy).
OPTIMISATION OF AN IN VITRO RAB PRENYLATION ASSAY TO DETECT THE ACTIVITY OF NANOMOLAR CONCENTRATIONS OF BISPHOSPHONATE DRUGS

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Rationale: Bisphosphonates drugs such as zoledronate target the skeleton and are used to inhibit bone resorption in diseases such as osteoporosis. Bisphosphonates act by preventing the synthesis of isoprenoid lipids necessary for the post-translational prenylation of small GTPase signalling proteins in osteoclasts. This results in cytosolic accumulation of the unprenylated forms of Ras-, Rho- and Rab-family GTPase proteins. Prenylation occurs via either of three prenyl transferases; farnesyl transferase, geranylgeranyl transferase 1 or geranylgeranyl transferase II (Rab GGTase), which transfer the prenyl group onto a carboxy-terminal cysteine residue.

Objective: We sought to optimise an in vitro prenylation assay in order to detect the effect of low concentrations of bisphosphonates on the prenylation of Rab proteins in cultured cells. Intracellular uptake of bisphosphonate results in the accumulation of unprenylated Rabs; incubation of cell lysates with recombinant RabGGTase and a biotinylated isoprenoid substrate analogue then results in transfer of the biotinylated lipid to unprenylated Rab proteins.

Methods and Results: J774.2 mouse macrophage cells were cultured with high concentrations of zoledronate (1-2 days treatment with 1-10µM, mimicking the concentrations achieved around osteoclasts in bone) or with low concentrations of zoledronate (>3 days’ treatment with 50-500nm, mimicking circulating levels that may be present in tissues outside the skeleton). Cell lysates were then used for the in vitro prenylation assay to biotinylate unprenylated Rab proteins. Acute treatment with high concentrations of zoledronate markedly inhibited Rab prenylation (ie high levels of biotinylated Rabs after in vitro prenylation). However, nanomolar concentrations of zoledronate as low as 50nM also caused a detectable inhibition of Rab prenylation in macrophages after 7 days’ treatment.

Conclusions: We show for the first time that effects of zoledronate as low as 50nM can be detected using this optimised prenylation assay. This furthers the likelihood that bisphosphonates affect cells other than osteoclasts, outside the skeleton.
IDENTIFICATION OF TUMOUR-ASSOCIATED MACROPHAGES AS TARGETS FOR THE ANTI-CANCER ACTIVITY OF BISPHOSPHONATE DRUGS

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Rationale: Bisphosphonates (BPs) such as zoledronic acid (Zometa; ZOL) have become the standard treatment for metastatic bone disease due to their ability to inhibit osteoclast-mediated bone resorption. The molecular target of ZOL in osteoclasts is FPP synthase. Inhibition of this enzyme prevents the synthesis of isoprenoid lipids necessary for the prenylation of essential small GTPases. BPs have anti-cancer effects in preclinical cancer models, independent of their effects on osteoclasts, and, more recently, have been shown to lead to increased survival in some clinical trials of patients with breast cancer and multiple myeloma. However, the exact mechanisms underlying these anti-cancer effects in vivo are still unknown.

Objective: The extent to which BPs are internalised in vivo by cells other than osteoclasts (especially other cells of the myeloid lineage) is unclear. We have used a novel, fluorescently-labelled BP (AF647-RIS), together with flow cytometry and 2-photon microscopy, to determine the cell types capable of internalising BP in the 4T1 murine breast cancer model.

Methods and Results: Within minutes of tail vein injection, intravital 2-photon imaging revealed the flow of BP into mammary tumours via the disorganised tumour vasculature, with slow diffusion and retention in tumour tissue. Within two hours F4/80+ tumour-associated macrophages (TAMs) were visualised to internalise the BP by endocytosis and phagocytosis. Flow cytometric analysis of the tumours 24hr later confirmed that uptake occurred predominantly by CD11b+F4/80+ macrophages, but not in tumour cells. BP did not accumulate in normal mammary tissue.

Conclusions: In summary, we provide conclusive evidence that BP can be internalized in vivo by TAMs completely outside the skeleton. Given the important role of TAMs in promoting tumour progression and metastasis, our studies suggest that the anti-tumour actions of BPs may be indirect and via myeloid cells such as tumour-associated macrophages.
**Anti-Sclerostin Treatment Preserves Bone Mass in a Murine Model of Multiple Myeloma**

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Multiple myeloma (MM) is a neoplastic disease of plasma cells associated with a progressive and highly destructive osteolytic bone disease, severe bone pain and pathological fractures. Suppression of osteoblastic bone formation contributes to the development of the lytic disease. While anti-sclerostin (anti-SOST) antibody is emerging as a potent bone anabolic agent in osteoporosis, it is unknown whether inhibiting sclerostin will prevent osteoblast suppression in MM. We aimed to explore the potential of anti-SOST treatment to preserve bone mass in a murine model of MM.

5TGM1eGFP murine myeloma cells were labeled with a membrane dye (DiD), retained by dormant, non-dividing cells, but lost as cells divide. These cells were injected (i.v) into C57BLKawRij mice, a well-established murine model of myeloma. Anti-SOST or control antibody was administered weekly, over a period of 4 weeks. Long bones were isolated for microCT and histomorphometric analyses. The spleen and bone marrow cells were also isolated to assess tumour burden by flow cytometry and CD138⁺ve cells by immunohistochemistry.

Anti-SOST treatment preserved bone mass, significantly increasing trabecular bone volume in both naïve and tumour burdened mice (p<0.05). Trabecular thickness and number were both increased (p<0.05). There was no effect of anti-SOST treatment on cortical bone volume in tumour bearing mice. Flow cytometric analyses of both the bone marrow and spleen demonstrated no effect of anti-SOST treatment on tumour burden. Immunologic grading in all tumour bearing mice confirmed extensive tumour burden. Histomorphometric analysis will determine the mechanism for the observed changes in bone volume through the analysis of parameters for bone formation and bone resorption.

This study demonstrates that while anti-sclerostin treatment is ineffective in controlling tumour burden in murine myeloma, it effectively preserved bone mass. Hence sclerostin antibody treatment demonstrates potential to address the suppressed bone formation seen in MM.
In vivo small molecule screening for novel osteogenic drugs

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Osteoporosis, a disease characterised by reduced bone density and increased risk of bone fracture is primarily treated with drugs that prevent bone loss (e.g. osteoclast inhibitors). As part of a complementary approach, we are using zebrafish embryos to identify new therapeutic drugs that stimulate bone formation. A transgenic zebrafish has been developed wherein a luciferase reporter is controlled by the expression of the osterix promoter, which is expressed in differentiating and mature bone building osteoblasts. Our preliminary data indicates that the osterix:Luciferase reporter responds appropriately to known osteoblast differentiation signals (e.g. RA and BMP). In combination with robust methods that we have developed for the quantification of luciferase expression from zebrafish embryos the osterix:Luciferase reporter enables high throughput screening of small molecule libraries with the goal of identifying novel osteoporosis therapeutics.
MCP-1 Gene Expression Is Specifically Regulated Following Stress Fracture Initiation, But Blocked By The Dominant Negative Mutant, 7ND.

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MCP1 (or CCL2) is a CC chemokine and plays a critical role in recruiting immune cell precursors. We saw that within 4 hours of stress fracture (SFx) initiation, MCP1 gene expression was significantly elevated, followed by increased serum levels within 24h. Specific inhibition of MCP1 would test the significance of its expression for bone cell recruitment. We hypothesise that a plasmid DNA encoding a dominant negative mutant of MCP1 (7ND) will inhibit its gene expression associated with SFx. SFx was created in the right ulna of wistar rats using cyclic end-loading. Unloaded animals were used as a control. 24 h prior to loading, 7ND plasmid vector was injected in the thigh muscle to overexpress 7ND protein, which was then secreted into systemic circulation. Rats were euthanized 4h after loading (n=5/group) and RNA extracted for quantitative real time PCR analysis using TaqMan assays. In untreated rats, there was ~33 fold increase (P<0.001) in MCP-1 expression 4h after loading. Treatment with 7ND abolished the loading related increase in MCP-1, with gene expression levels lower than un-loaded control rats. We hypothesise that activation of the remodelling phase of SFx repair will be inhibited following this suppression of MCP-1. Because MCP-1 is markedly upregulated by SFx and by PTH, we propose that it provides important regulation of chemotaxis and osteoclast differentiation during initiation events of bone remodelling.
Pre-clinical treatments for fracture repair in neurofibromatosis type 1

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Aims of the study: Congenital pseudarthrosis of the tibia (CPT) is a severe orthopaedic complication of the genetic condition, Neurofibromatosis type 1 (NF1) which develops following fracture of dysplastic tibiae. Healing is recalcitrant, involving multiple surgical interventions and amputations. In light of previous preclinical studies in a simplistic bone model of NF1, we hypothesised that recombinant human bone morphogenetic protein-2 (rhBMP-2) co-treated with a bisphosphonate (zoledronic acid/ZA) would improve bone union and reduce fibrosis tissue in a mouse model of Nf1-/- pseudarthrosis featuring high non-union, poor new bone formation, excessive osteoclast-driven bone resorption and invasion of mesenchymal/fibrous tissue.

Methods: Fractures were induced in Nf1flox/flox mice and double inactivation of the Nf1 locus was induced via transduction with a Cre-expressing adenovirus, as previously published. Interventions included 10µg local rhBMP-2 via collagen sponge and 5 doses of 0.02mg/kg systemic ZA starting from 3 days post-op (n=15/group). Outcome measures 21 days post-fracture were radiographic union rate (Faxitron XR), microCT (Skyscan 1174); descriptive histology; and mechanical testing (Instron).

Results: Animals treated with rhBMP-2/ZA showed the highest rate of bone union (93%) compared to vehicle (7%), ZA (0%), and rhBMP-2 alone (86%) (*p<0.0001). Treatment with rhBMP-2 produced a 2-fold greater increase in BV compared to ZA (p<0.0001) and a 3-fold increase compared to vehicle (p<0.0001). Co-treatment with rhBMP-2/ZA led to significant increases in BV compared to vehicle**, ZA** and rhBMP-2** (**p<0.0001). Callus fibrous tissue was decreased with rhBMP-2/ZA co-treatment vs. vehicle and rhBMP-2 groups.

Conclusion: This data demonstrates the utility of our pre-clinical model for screening therapies for NF1/CPT and supports the advancement of clinical trials to assess the efficacy of rhBMP-2/ZA combination therapy. Our results suggest that an approach that does not specifically target the deficient pathways in NF1 can still be effective in promoting fracture repair.
A determination of the rate of burial of osteoblasts in bone matrix during osteonal infilling

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Introduction. The burial of osteoblasts in bone matrix during formation is a complex process that remains poorly understood. Osteoblast burial determines the density of osteocytes, but it is also regulated by the expanding network of underlying osteocytes. Here, new measurements of osteocyte lacuna densities are used to get insights into the regulation of osteoblast burial in human cortical osteons.

Aims. (i) To determine how osteoblast burial rate varies during osteonal infilling; (ii) To determine the correlation between burial rate and the population of pre-existing osteocytes.

Materials & Methods. A mathematical model was developed to relate osteocyte density with osteoblast density, matrix synthesis rate, osteoblast burial rate, and bone substrate curvature. The equations were solved for osteoblast burial rate to allow its estimation from experimental data. In particular, osteocyte lacuna density in human osteons was determined from synchrotron-radiation microCT scans of cortical bone samples from a 20yr old male (Melbourne Femur Collection).

Results. The density of osteocytes generated at the moving deposition front depends solely on the ratio of the instantaneous burial rate and matrix synthesis rate – it is remarkably independent of osteoblast density and substrate curvature. Consequently, burial rate can be estimated from osteocyte density and matrix synthesis rate only. Using our estimates of osteocyte density in osteons, burial rate was found to decrease during osteonal closure (see Figure). Burial rate correlated positively with the density of osteocytes close to the deposition front, and negatively with the total number of osteocytes present underneath.

Conclusions. Marotti hypothesised that osteocytes promote osteoblast burial when they become covered with sufficient new matrix. The positive correlation between burial rate and osteocyte density near the front is inconsistent with this hypothesis. However, our results suggest that such a control of osteoblast burial could emanate as a collective signal from a larger group of osteocytes.
Clinical Decision Support Computer Software for Osteoporosis and Fracture Prevention

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The large gap between what is recognised as optimum treatment of osteoporosis and what is actually received by patients exists in all countries. In Australia only 30% of those who suffer a minimal trauma fracture are investigated for osteoporosis and receive treatment. The increasing use of computerised patient records offers new opportunities to aide clinical decision making and improve patient health outcomes.

We have developed a Fracture and Osteoporosis Investigation and Treatment (F.R.O.I.T) software tool. Uniquely, it is designed to be integrated with existing patient data software so the tool can be pre-populated with patient data and can be used the time of consultation between the doctor and the patient. With this software tool it is possible to increase rates of investigation, assist management decisions and improve patient understanding of fracture risk.

Early utility testing with general practitioners has proved favorable. General practitioners surveyed were most impressed with the individualized graphic display of fracture risk based on the Garvan Fracture Risk Calculator. There is large public awareness of chronic medical conditions such as heart disease and diabetes. This awareness is less so for osteoporosis and the consequences of fracture so communication of risk may be particularly important for osteoporosis.

Electronic clinical decisions support systems are one of the most promising interventions to improve uptake of guideline-based recommendations in clinical practice. A recent systematic review of randomised controlled trials of clinical decision support for chronic medical conditions found two thirds demonstrating improvement in clinical decision making. The F.R.O.I.T software tool is one of the first in the world to provide this type of clinical decision support for osteoporosis.

*Individual testing of the software tool will be available.*
Antibody to LRP6 is anabolic in C57Blk6/KaLwRij mice

Jenny Down, Michelle McDonald, Jessica Pettitt, Peter Croucher

Aims - LRP6 is a transmembrane cell surface receptor crucial to canonical Wnt/β-catenin signalling. Wnt signalling stimulates osteoblast differentiation and proliferation such that antibodies to antagonists of this pathway, such as sclerostin and DKK1, increase bone mass. Anti-LRP6 antibodies potentiate Wnt signalling through promotion of specific Wnt ligands. We hypothesized that Anti-LRP6 will increase bone mass in C57Blk6/KaLwRij naïve mice, through promoting bone formation. C57BLK6/KaLwRij mice are receptive to 5TGM1 model of multiple myeloma which causes bone loss and lytic lesions. Promoting anabolism through anti-LRP6 treatment may rescue the bone loss associated with this disease.

Method – Eleven male 7 week old C57BLK6/KaLwRij mice were injected iv with either 10mg/kg anti-LRP6 or control antibody once a week for 4 weeks. Seven days after the last injection the mice were culled and tibiae were isolated and for MicroCT analysis and decalcified for paraffin histology. Non-consecutive TRAP sections were analysed for osteoblast and osteoclast parameters.

Results – MicroCT analysis revealed a significant increase in BV/TV (p=0.001), trabecular thickness (p<0.005) and trabecular number (p<0.001) with anti-LRP6 treatment compared to control antibody treated mice. There was also a significant increase in BV (p<0.005) in the cortex. Histomorphometric analysis showed no significant differences between anti-LRP6 and control antibody in osteoblast or osteoclast parameters for trabecular or cortical bone. There was a significant increase in bone area (p=0.001) and bone perimeter (p<0.002) of trabecular bone, similar to that seen with uCT analysis.

Conclusion – Anti-LRP6 antibody significantly increased both trabecular and cortical bone volume in naïve mice. Osteoblast and osteoclast parameters were not significantly altered with anti-LRP6, suggesting that the cellular effects of this agent are undetectable after 4 weeks of treatment. The anabolic effect of this LRP6 antibody may rescue the bone loss in C57Blk6/KaLwRij 5TGM1 bearing mice, providing a novel therapy for tumour associated bone disease.
ASSESSING BONE RESPONSE TO OVARIECTOMY IN THE MYELOMA PERMISSIVE C57/Bl6KalwRij MOUSE

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The C57/Bl6KalwRij mouse strain is permissive to myeloma bone disease development after inoculation with 5TGM1 murine myeloma cells. We are interested in how ovariectomy (OVX) induced alterations in bone turnover will impact myeloma cell colonisation and growth in bone. To perform these investigations, analysis of OVX induced bone response in C57/Bl6KalwRij mice was required. The aim of this study was to assess the time course of bone loss following OVX using MicroCT analysis.

Ten week old female C57/Bl6KalwRij mice underwent ovariectomy or sham surgery with harvest at 1, 3 or 4 weeks post-surgery. Tibiae were MicroCT scanned at a resolution of 4.3um using the skyscan 1172. Analysis of trabecular and cortical bone was performed in the proximal metaphysis.

At 1 and 3 weeks post OVX, BV/TV was not different between OVX and Sham groups. However, by 4 weeks, the OVX group showed a 40% reduction in trabecular BV/TV compared to Sham (p<0.01). This was as a result of a 34% reduction in TbN (p<0.01) and an 8% reduction in TbTh (p<0.05). Cortical bone volume was not altered at each time point examined. Interestingly, both the Sham and OVX groups showed a reduction of 31% (p<0.01) and 41% (p<0.01) respectively in Tb BV/TV between 1 and 3 weeks post-surgery. These changes were due to reduced TbN.

In conclusion, significant trabecular bone loss was not detected until 4 weeks post OVX. The significant reduction in trabecular number suggests this was a result of increased bone resorption, however histological analysis of the cellular mechanisms at the time points examined will confirm this. Interestingly, the sham procedure also led to bone loss between 1 and 3 weeks post-surgery, confirming the necessity for surgical control groups in our future myeloma experiments.
LONG-TERM EFFECTS OF BISPHOSPHONATE THERAPY IN CHILDREN WITH OSTEOGENESIS IMPERFECTA

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Bisphosphonate therapy is the mainstay of medical treatment in osteogenesis imperfecta (OI). There are varying treatment practices but limited information on long-term effects. The aims of this study were to evaluate the clinical outcomes of intravenous bisphosphonate treatment in children with moderate-severe OI who had progressed from active to maintenance therapy for greater than two years.

A retrospective review was conducted on 17 patients with moderate-severe OI. Clinical data, fracture history, biochemistry, dual energy x-ray absorptiometry (DXA) parameters, vertebral measurements and metacarpal cortical thickness were collected at three time points: (1) Before treatment, (2) following active treatment with high dose bisphosphonates (zoledronate 0.05mg/kg six-monthly or pamidronate 6-9mg/kg/year) and (3) after establishment on a low dose maintenance treatment phase (zoledronate 0.025mg/kg six-monthly or pamidronate <4mg/kg/year).

The mean age at commencement of active treatment was 4.8±2.6 years and maintenance therapy was 10.0±2.6 years. Mean time on maintenance therapy was 4.1±1.4 years. Height Z-scores did not change significantly over time but weight z-scores during active and maintenance treatment were higher than pre-treatment levels (see Table). There was a significant reduction in fracture rate on active treatment. Biochemical analysis of bone homeostasis revealed a significant reduction in bone turnover markers between active and maintenance treatment. DXA showed a significant improvement in bone mineral density (BMD), bone mineral content (BMC) and BMC for lean-tissue mass Z-scores. Vertebral height increased in both normal lumbar vertebrae (L1-L4) and fractured thoracic and lumbar vertebrae from pre-treatment to active therapy and was maintained during maintenance treatment. Assessment of hand x-rays showed that 2nd metacarpal cortical thickness and relative cortical area increased over the treatment periods.

Maintenance intravenous bisphosphonate therapy preserved the beneficial effects of a high dose active treatment regimen. Further studies are required to determine the optimal bisphosphonate treatment regimen in the management of children with OI.

<table>
<thead>
<tr>
<th></th>
<th>Pre-treatment</th>
<th>Active</th>
<th>Maintenance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height Z-score</td>
<td>-1.4 ± 1.6</td>
<td>-1.3 ± 1.7</td>
<td>-1.7 ± 2.4</td>
</tr>
<tr>
<td>Weight Z-score</td>
<td>-1.2 ± 1.6</td>
<td>0.1 ± 1.5 *</td>
<td>0.0 ± 1.6 *</td>
</tr>
<tr>
<td>Fracture rate (number/year)</td>
<td>1.3 ± 1.1</td>
<td>0.7± 0.7 *</td>
<td>0.7 ± 0.9 *</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>2.43 ± 0.10</td>
<td>2.35 ± 0.07</td>
<td>2.30 ± 0.07</td>
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<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>277 ± 63</td>
<td>210 ± 46 *</td>
<td>164 ± 74 *</td>
</tr>
<tr>
<td>Phosphorus (mmol/L)</td>
<td>1.61 ± 0.17</td>
<td>1.50 ± 0.13 *</td>
<td>1.40 ± 0.15 *</td>
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<tr>
<td>Osteocalcin (nmol/L)</td>
<td>7.4 ± 3.7</td>
<td>7.8 ± 6.2</td>
<td>3.4 ± 3.0 *</td>
</tr>
<tr>
<td>25-OH-Vitamin D (nmol/L)</td>
<td>75 ± 32</td>
<td>69 ± 22</td>
<td>62 ± 17</td>
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<tr>
<td>Urine Deoxypyridinoline:Cr ratio</td>
<td>115 ± 75</td>
<td>117 ± 89</td>
<td>57 ± 52</td>
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<tr>
<td>(nM/mM)</td>
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<tr>
<td>Total BMD Z-score</td>
<td>-0.9 ± 1.2</td>
<td>-0.6 ± 1.2 *</td>
<td>-0.8 ± 1.8</td>
</tr>
<tr>
<td>L1-L4 BMD Z-score</td>
<td>-2.5 ± 1.2</td>
<td>-0.5 ± 1.3 *</td>
<td>-0.3 ± 1.1 *</td>
</tr>
<tr>
<td>Total BMC Z-score</td>
<td>-1.1 ± 0.6</td>
<td>-0.7 ± 1.5 *</td>
<td>-0.9 ± 1.9 *</td>
</tr>
<tr>
<td>BMC for LTM Z-score</td>
<td>-1.4 ± 1.1</td>
<td>0.2 ± 1.7 *</td>
<td>0.5 ± 1.5 *</td>
</tr>
<tr>
<td>Vertebral height (anterior:length ratio)</td>
<td>0.70 ± 0.06</td>
<td>0.76 ± 0.07 *</td>
<td>0.76 ± 0.09 *</td>
</tr>
<tr>
<td>2nd Metacarpal relative cortical area</td>
<td>8.6 ± 4.0</td>
<td>14.6 ± 4.9 *</td>
<td>21.8 ± 6.5 *</td>
</tr>
</tbody>
</table>

*Table: Mineral homeostasis, DXA and bone morphometry data*
Values represent mean±SD, asterisk represents p<0.05 compared to pre-treatment values